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Integrated assessment of lifestyle habits and pharmacological intervention in routine clinical care

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# Opportunities for improvement of cardiovascular risk management in patients with type 2 diabetes and chronic kidney disease

## Integrated assessment of lifestyle habits and pharmacological intervention in routine clinical care

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# PART I

Integrated assessment of pharmacological and lifestyle treatment in routine cardiovascular risk management of patients with type 2 diabetes in secondary care

# CHAPTER 1

## Introduction
and aims

# Introduction

Cardiovascular disease (CVD) is one of the most important disorders worldwide, 31% of mortality can be attributed to CVD. In the Western world, the development and implementation of cardiovascular risk management has initiated a trend towards lower CVD prevalence and mortality in the last decades[1]. However, CVD still causes substantial morbidity and mortality, and CVD prevalence may even increase once again, due to the alarming rise of obesity, diabetes and physical inactivity, in addition to aging and population growth[2,3]. Therefore, CVD prevention is of the utmost importance in modern healthcare.

CVD is as old as humanity itself: using computed tomography, signs of atherosclerosis have been found in ancient Egyptian mummy's (1981 BCE and 334 CE)[4]. Study of CVD started in 1628, after the publication of "Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus" (translation "An Anatomical Exercise on the Motion of the Heart and Blood in Living Beings") by William Harvey, in which he introduced the concept of closed loop blood circulation. Since the beginning of the 20th century, there has been a steep rise in the average life-expectancy in the Western World, from 45 years in 1900 to 81 years in 2015[5,6], mostly due to improved perinatal care and decline in mortality of infectious diseases. This increase in life expectancy was paralleled by a dramatic increase in CVD-related mortality in Europe. Formal research into the epidemiology of CVD started after the second world war, at which time CVD was labelled as an epidemic for the first time. Moreover, since the early 20th century, after the invention of coronary artery catheterization and the electrocardiogram, understanding of heart disease markedly increased. In the 1970's, multiple lifestyle and biological risk factors for CVD were firmly established, such as diabetes, hypertension, smoking and a diet rich in salt and saturated fats. By then, it had become clear that lifestyle changes in Western countries towards physical inactivity, an unhealthy diet and smoking played a pivotal role in the increasing prevalence of CVD. Strategies to prevent CVD became a core issue in health care.

In the current day and age, we have gathered substantial knowledge on the pathogenesis, risk factors and treatment of CVD, and since the 1980's there has been a trend towards lower CVD prevalence and mortality in Europe[1]. However, the alarming ongoing rise of obesity, diabetes and physical inactivity, in addition to aging and population growth, could once again result in an increase in CVD mortality if no appropriate measures are taken[2,3]. Because of the high morbidity, mortality and costs associated with CVD, preventing cardiovascular disease remains a major aim of healthcare worldwide. Primary prevention is defined as measures to reduce the occurrence of new-onset disease. Secondary prevention is defined as measures to mitigate the clinical course and outcomes

in patients. As such, primary prevention of CVD is done from a public health perspective, with the goal of reducing new-onset CVD in the general population, for example by reducing sodium content in bread or anti-smoking campaigns. In addition, in subjects without previous CVD events and without diagnosed high-risk diseases, primary prevention is done in primary health care, using lifestyle and pharmacological intervention to reduce the risk of a first CVD event. Secondary CVD prevention is done in patients already diagnosed with one or more high-risk diseases, (i.e. type 2 diabetes mellitus (T2DM); chronic kidney disease (CKD); previous CVD), who have a very high risk to develop CVD within 10 years. In secondary CVD prevention, patients are already part of routine clinical care, therefore preventive measures are applied face-to-face, and, theoretically, can be fitted to the needs and desires of the individual patient[7,8]. In this thesis, we study secondary prevention of CVD in patients with T2DM and CKD.

### Risk factors and guidelines

Risk factor management has become the major issue in primary and secondary CVD prevention. CVD is associated with the presence of one or more risk factors, which can either be non-modifiable (i.e. age, gender ethnicity, family history of CVD), or modifiable (Table 1). Modifiable risk factors are by far the largest contributors to CVD risk. The INTERHEART study demonstrated that the following nine modifiable risk factors accounted for 90% of myocardial infarctions worldwide: lipid abnormalities, smoking, hypertension, diabetes, abdominal obesity, psychosocial factors, low consumption of fruits, vegetables, and alcohol, and physical inactivity[9]. Moreover, it has been estimated that with adequate intervention on modifiable risk factors, approximately 80% of all CVD can be prevented[10]. Therefore, it is of great importance that modifiable risk factors are identified and treated in high-risk patients.

Epidemiological knowledge on the role of risk factors is translated to protocols for routine clinical care in cardiovascular risk management (CVRM) guidelines. In CVRM guidelines for primary and secondary prevention, target values for each modifiable risk factor are incorporated, together with treatment strategies to achieve these target values[11-13]. The first step in these CVRM is always lifestyle management, followed by pharmacological treatment.

### Lifestyle

Webster's dictionary defines lifestyle as "the typical way of life of an individual, group or culture". In medicine, lifestyle often refers to behaviours regarding diet, physical activity, sleeping and psychological stress. Since the late 1940's, lifestyle in the Western world is increasingly characterized by sedentary behaviour, physical inactivity, smoking, alcohol abuse and a diet rich in refined sugar, saturated fats and salt and low in vegetables, fruits

and whole grains. In parallel, there has been an alarming and steady rise in the prevalence of obesity; the worldwide prevalence of obesity nearly tripled between 1975 and 2016[14]. In 2014, one in three people was overweight, and one in ten people was obese. Western lifestyle has been associated with the pathogenesis of many diseases, among which type 2 diabetes, cardiovascular disease, multiple types of cancer and auto-immune diseases[15-17]. It is unsurprising that along with obesity, the prevalence of lifestyle related health problems has also risen in the last decades. The global prevalence of diabetes, for example, has risen from 5% in 1980 to 9% in 2014, and in the Eastern Mediterranean region these numbers were even more alarming: from 6% to 14%[18].

**Table 1.** Non-modifiable and modifiable risk factors in cardiovascular risk management

| *Non-modifiable risk factors* | |
|---|---|
| Male gender | |
| Age | |
| Ethnicity | |
| Family history of CVD | |
| Previous CVD | |
| *Modifiable risk factors* | |
| *General* | *Pharmacologically treatable risk factors* |
| Unhealthy diet | Hypertension |
| Physical inactivity | Dyslipidaemia |
| Obesity | HbA1c |
| Smoking | |
| High alcohol intake | |
| Psychological stress | |
| Low socioeconomic status | |

CVD, cardiovascular disease

Because lifestyle is a key modifiable risk factor in the prevention of CVD, lifestyle change is always the first step to reduce CVD risk in treatment guidelines. For the general population, smoking cessation and a body mass index ≤25 kg/m2 is advised. Target values for diet and exercise can differ between guidelines. In the Netherlands, the Health Council has provided guidelines for a healthy diet and for physical activity based on available evidence (Table 2)[19,20], and both guidelines are regularly updated.

**Table 2**. Lifestyle guidelines formulated by the Dutch Health Council

| | |
|---|---|
| *General* | |
| Smoking cessation | |
| Body Mass Index ≤25kg/m$^2$ | |
| *Physical activity* | |
| Physical activity is good for you – the more, the better | |
| ≥150 minutes physical activity per week of at least moderate intensity, spread over several days | |
| Twice a week bone and muscle strengthening exercise; older people should combine this with balance exercises | |
| *Diet* | Follow a dietary pattern that involves eating more plant-based and less animal-based food |
| Vegetables | ≥200 g/day |
| Fruit | ≥200 g/day |
| Wholegrain products | ≥90 g/day |
| Legumes | ≥1 portion/week |
| Unsalted nuts | ≥15 g/day |
| Dairy products | A few portions/day (including milk or yoghurt) |
| Fish | ≥1 portion/week |
| Black or green tea | 3 cups/day |
| Cereal products | Replace refined cereal products by whole-grain products |
| Cooking fats | Replace butter, hard margarines, and cooking fats by soft margarines, liquid cooking fats, and vegetable oils |
| Coffee | Replace unfiltered coffee by filtered coffee |
| Meat | Limit the consumption of red meat, particularly processed meat |
| Sugar containing beverages | Limit consumption |
| Alcohol | ≤1 glass/day |
| Salt | ≤6 g/day |

Successful lifestyle adjustment can prevent or even reverse the development of T2DM, hypertension and dyslipidaemia, and can reduce overall risk of CVD[21-24]. Moreover, in patients with established disease (T2DM, CKD, previous CVD), lifestyle intervention can also reduce the necessary number of pharmacological agents[25-27]. In a randomized study in primary care, a higher percentage of weight loss was stepwise associated with higher degree of T2DM remission, varying between with 0% remission in those with weight gain, and 86% remission in those with ≥15% weight loss[28]. In contrast, insulin treatment induces weight gain and therefore eventually increases insulin resistance, causing a vicious circle[29]. In a meta-analysis, Patients with T2DM who underwent bariatric surgery, which significantly reduced weight, had a 5.9 times higher rate of diabetes remission in a 5-year period, compared to patients in regular T2DM care[30]. Although bariatric

surgery is a surgical intervention, and not a lifestyle adjustment, these data serve as a proof of principle that weight loss can induce diabetes remission. Apart from weight loss, increasing physical activity can have beneficial effects on glycaemic regulation, lipids, blood pressure, cardiovascular events, mortality, and quality of life[31]. In addition, dietary intervention can improve glycaemic control, blood pressure, and dyslipidaemia[32,33]. Finally, smoking cessation is associated with a 36% risk reduction in overall mortality and 32% reduction in non-fatal myocardial infarction, next to other health benefits[34].

### Pharmacological intervention and treatment targets

After lifestyle adaptation, pharmacological intervention is used as the second step in management to reduce blood pressure, LDL-cholesterol and, in T2DM, HbA1c. In addition, in those with previous CVD, the use of different types of anticoagulants (i.e. antiplatelet drugs, coumarin derivatives, new oral anticoagulants) is considered. Pharmacological treatment is considered when target values for these parameters are not met by lifestyle adjustment, or when the 10-year CVD risk is high (>5%; such as in T2DM and CKD)[35,36].

Hypertension is one of the most important risk factors for CVD. In the United States, high blood pressure was the second most accountable risk factor for CVD, with smoking being the first[37]. A large meta-analysis showed that 20 mmHg higher systolic blood pressure and 10 mmHg higher diastolic blood pressure were each associated with a doubling in the risk of death from stroke, heart disease, or other vascular disease[38]. The target for blood pressure reduction has gradually become more stringent in the last decade. In earlier guidelines, a target of <140/85-90 mmHg has been recommended[36,39]. However, after new evidence has shown additional beneficial effects of more intensive blood pressure treatment[40,41], the most recent guideline from the American Heart Association recommended a target of <130/80 mmHg in high-risk patients[42]. Blood pressure reduction is associated with a very large risk reduction for CVD: meta-analyses demonstrated a hazard ratio for major cardiovascular events of 0.36 (95% CI 0.26, 0.51) in patients who achieved 120-124 mmHg compared to patients with >160 mmHg[40]. Blood pressure can be pharmacologically treated by inhibition of the renin-angiotensin-aldosterone system (RAASi) with angiotensin converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB); thiazide, loop and potassium saving diuretics; alpha and/or beta blockers; and calcium antagonists. In the case of albuminuria, either in T2DM or CKD, RAASi is preferred as it protects against renal function decline and reduces CVD risk.

Treatment of dyslipidaemia, characterized by high LDL cholesterol and low HDL cholesterol, also plays a pivotal role in CVD prevention. LDLc reduction is achieved mainly through statin treatment. It has repeatedly been shown that statin treatment does not only reduce LDLc, but also reduces the risk of CVD in primary and secondary preven-

tion[43,44]. Every 1.0 mmol/l reduction in LDLc is associated with a corresponding 20-25% reduction in CVD mortality and non-fatal myocardial infarction[45]. In the Netherlands, the target LDLc value is set at ≤2.5 mmol/l. It should be noted that because of the pleiotropic beneficial effects of statin treatment, according to the latest literature statin treatment is indicated in all high-risk patients, regardless of baseline LDLc[46].

Finally, in patients with T2DM, adequate glycaemic control (i.e. HbA1c) has been shown to substantially reduce the risk of microvascular complications (i.e. nephropathy, retinopathy and neuropathy), and to a lesser extent also of cardiovascular events, albeit that the preventive effects of the latter only become apparent after many years[47-49]. Each 1% of mean HbA1c reduction has been associated with risk reduction of 21% for any diabetes-related complication[48]. On the other hand, too strict glycaemic control (target HbA1c <42 mmol/mol) has been associated with increased mortality, despite a reduction in CVD risk[50]. Therefore, in clinical guidelines the target of HbA1c <53 mmol/mol has been incorporated[36,51]. It should be noted that the American Diabetes Association and the European Association for the Study of Diabetes have released a joint statement that HbA1c target should be individualized per patient, based on age, frequency of hypoglycaemia and life-expectancy among other things[52]. The first step of blood glucose lowering treatment is usually metformin, followed by addition of another oral blood glucose lowering drug, glucagon-like peptide-1 analogues, or a basal insulin regimen[36]. As different classes of blood glucose lowering drugs have different effects on total blood glucose lowering, weight gain and risk of hypoglycaemia, for each individual patient benefits and disadvantages of each option should be considered. When the target HbA1c is not reached, blood glucose lowering therapy can be intensified stepwise until the final step of a basal/bolus insulin regimen[36].

### Low achievement of CVRM treatment targets in clinical practice

As stated before, theoretically 80% of all CVD can be prevented by treating known modifiable risk factors[10]. However, in clinical practice such a risk reduction is rarely achieved, mainly due to the fact that targets for blood pressure, LDL cholesterol and glycaemic control are not reached in a large number of patients [53-58]. Of note, very little data is available on to which extent lifestyle targets are reached. Improving target achievement in clinical practice could lead to an important reduction in CVD, especially in patients already at high risk. Moreover, the reason why blood pressure, LDLc and HbA1c targets are not reached is largely unknown, yet low target achievement is often attributed to low patient adherence to pharmacological and lifestyle treatment, physician inertia to target non-achievement and deficiencies of healthcare systems[39]. Therefore, a closer look on the implementation of CVRM in clinical practice is warranted, so that opportunities to improve target achievement can be identified.

### CVRM in routine care: the accent lies on pharmacological treatment

In the Netherlands, CVRM screening and management is initially performed by general practitioners in primary health care. In general, patients are encouraged to stop smoking and adopt a healthy lifestyle. If a patient develops hypertension, dyslipidaemia or glucose intolerance, lifestyle intervention is the first step of treatment, including referral to a dietician[13]. However, only three hours of dietician guidance per year is reimbursed by health care insurance, therefore long-term follow up is not possible. Reimbursement for participation to programs designed to increase physical activity is only covered by some insurance companies, and always requires additional health care coverage. Smoking cessation programs are reimbursed by many insurances, with a maximum participation of one program per year. Pharmacological treatment, which is invariably reimbursed, is initiated when risk factors persist despite lifestyle intervention, or when patients have a very high risk of developing CVD (>10%).

Patients are referred to secondary health care in the case of treatment resistant hypertension, development of CVD, renal function impairment, or complex T2DM (macroalbuminuria, difficulty to reach the target HbA1c). In secondary health care, patients often have progressed disease or have complex comorbidity, and therefore polypharmacy is common. By then, the majority of CVRM consists of monitoring pharmacologically treatable risk factors, i.e. blood pressure, dyslipidaemia and HbA1c, and adjusting the, often abundant, pharmacological regimen when appropriate. The role of lifestyle in secondary health care is not well defined. Monitoring of lifestyle in the clinical care setting is rarely performed. In addition, reimbursement for lifestyle care is similar as in primary care, three hours of dietician guidance per year, and therefore is insufficient to provide continuous intervention.

### Opportunities for improving CVRM

To identify opportunities for improving CVRM in secondary health care, it is important to study how well lifestyle and pharmacological guidelines on risk factor management are incorporated into clinical care. For pharmacological management, there are established stepwise algorithms for (intensification of) treatment. Additionally, prescription of treatment and effects of treatment can be closely monitored. Treatment compliance could be measured as well, but is difficult to do objectively, and therefore is not often done in routine care. Accordingly, we have some insight into the application of pharmacological treatment in secondary health care. However, for lifestyle treatment, frequency of prescription, compliance and results of treatment in routine care are largely unknown. Sub-optimal lifestyle management might be a missed opportunity in CVRM, especially in secondary prevention where patients regularly visit the outpatient clinic and pharmacological interventions are already tailored to the individual patient. Therefore, there is

a need to study lifestyle and pharmacological treatment of patients in secondary health care and investigate the opportunities to improve CVD prevention. To this end, we initiated the DIAbetes and LifEstyle Cohort Twente (DIALECT). In this cohort we perform objective measurements of lifestyle in patients with T2DM treated in secondary health care, and also gather extensive data on clinical condition, pharmacological treatment, and biochemical investigations.

### New opportunity in CVRM: neurohumoral activation

Optimizing pharmacological treatment is another opportunity for improving CVRM in routine clinical care. By studying interindividual differences in pathophysiological pathways behind increased CVD risk, the most adequate pharmacological agents can be selected for the individual patient. One of such pathophysiological pathways is excess neurohumoral activation[59,60]. The hormone aldosterone has been proposed to have a detrimental effect on cardiovascular and renal health[61,62]. Aldosterone is stimulated by angiotensin II (volume depletion), potassium (hyperkalaemia) and adrenocorticotropic hormone, and stimulates volume retention and potassium wasting. In the last few decades it has become clear that aldosterone also exerts pro-inflammatory and pro-fibrotic effects on multiple target organs, such as the heart, the kidney, the vasculature and adipose tissue[61-63]. These effects are applied through binding of aldosterone to its receptor, the mineralocorticoid receptor (MR), or without binding to the MR (non-genomic effects) [64,65]. Inhibition of aldosterone by the use of MR antagonists leads to a reduction of CVD in heart failure patients and has shown to be effective in treatment-resistant hypertension[66-69]. In CKD and T2DM, MR antagonists might have the potential to further reduce residual albuminuria during renoprotective RAASi[70]. This is supported by the finding that during RAASi, in 50% of patients aldosterone levels return to or even proceed pre-treatment levels, a phenomenon known as aldosterone breakthrough[71]. However, it is unknown whether aldosterone breakthrough is also associated with the pro-inflammatory and pro-fibrotic effects of aldosterone. Currently the role of aldosterone and MR antagonism in CVRM is unclear, and more research is needed to elucidate the mechanisms behind aldosterone regulation in health and disease.

Additionally, excess exposure to cortisol might play an important role in the development of CVD risk factors. The phenotype of hypercortisolaemia, also known as Cushing's syndrome, has many similarities with the metabolic syndrome and T2DM: central obesity, hypertension, dyslipidaemia, and insulin resistance. Relative hypercortisolism, increased cortisol exposure without overt high circulating levels of cortisol, has been proposed to play a role in the development and course of T2DM and CKD[59]. One mechanism by which cortisol exposure might be increased in these diseases, is through increased intracellular cortisol production. The enzyme 11beta-hydroxysteroid dehydrogenase type

1 (11β-HSD1), which is located in the liver and adipose tissues, converts inactive cortisone to active cortisol. Overactivity of 11β-HSD1 has been reported in overweight patients with T2DM, compared to those without T2DM[72,73]. Additionally, patients with renal function impairment, without T2DM, were found to have increased 11β-HSD1 activity[74,75]. Its counterpart, 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2) converts active cortisol into inactive cortisone. 11β-HSD2 is located in tissues rich which the MR, mostly in the kidney, and prevents binding of cortisol to the MR. Dysfunction of 11β-HSD2 results in the syndrome of apparent mineralocorticoid excess (SAME), of which excess liquorice consumption is a commonly known cause. Lower activity of 11β-HSD2 has also been reported in T2DM and CKD[72,76]. Therefore, both in T2DM and in CKD, 11β-HSD activities might be shifted towards higher intracellular cortisol production. Consequently, in both these condition, activities of 11β-HSD1 and 11β-HSD2 might play an important role in CVD prevention.

Taken together, data on regulation of aldosterone and 11β-HSD activities could pinpoint opportunities to optimize pharmacological treatment of excess neurohumoral activation (i.e. MR antagonists and 11β-HSD1 inhibitors) in T2DM and CKD.

# Outline and aims of the thesis

Cardiovascular risk management in routine clinical care does not reach its full potential of reducing CVD risk by 80%. We aim to study opportunities to improve CVRM in secondary prevention.

In **PART 1** of this thesis, we focus on the integrated role of lifestyle and pharmacological management in routine CVRM in patients with T2DM treated in secondary health care. To this end, we initiated the DIAbetes and LifEstyle Cohort Twente (DIALECT), in which we study target achievement, and opportunities in lifestyle and pharmacological treatment to improve target achievement, of blood pressure **(CHAPTER 2)** LDL cholesterol **(CHAPTER 3)**, and HbA1c **(CHAPTER 4)**. Additionally, we study the association between different markers of magnesium status (magnesium intake, 24h urinary magnesium excretion and plasma magnesium concentration) and prevalent coronary heart disease in **CHAPTER 5**. In **CHAPTER 6** we demonstrate the importance of objective lifestyle measurement, by comparing subjective and objective assessment of physical activity.
**PART 2** consists of in-depth studies on neurohumoral activation in patients with chronic kidney disease and T2DM, to improve knowledge on pathophysiological pathways behind increased CVD risk. First, we review available data on aldosterone and mineralocorticoid receptor antagonism in **CHAPTER 7**. Next, we investigate gender differences in aldosterone in healthy normotensive adults **(CHAPTER 8)**. In **CHAPTER 9** we study the association between renal function and aldosterone in a clinically relevant setting of chronic kidney disease, namely during RAASi. Lastly, in **CHAPTER 10** we compare 11β-HSD activities between patients with T2DM and health controls, and study the association between renal function and intracellular cortisol production by 11β-HSDs in T2DM.

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# CHAPTER 2

## Integrated Assessment of Pharmacological and Nutritional Cardiovascular Risk Management: Blood Pressure Control in the DIAbetes and LifEstyle Cohort Twente (DIALECT)

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# Abstract

Cardiovascular risk management is an integral part of treatment in Type 2 Diabetes Mellitus (T2DM) and requires pharmacological as well as nutritional management. We hypothesize that a systematic assessment of both pharmacological and nutritional management can identify targets for the improvement of treatment quality. Therefore, we analysed blood pressure (BP) management in the DIAbetes and LifEstyle Cohort Twente (DIALECT). DIALECT is an observational cohort from routine diabetes care, performed at the ZGT Hospital (Almelo and Hengelo, The Netherlands). BP was measured for 15 minutes with one-minute intervals. Sodium and potassium intake was derived from 24-hour urinary excretion. We determined the adherence to pharmacological and non-pharmacological guidelines in patients with BP on target (BP-OT) and BP not on target (BP-NOT). In total, 450 patients were included from August 2009 until January 2016. The mean age was 63 ± 9 years, and the majority was male (58%). In total, 53% had BP-OT. In those with BP-NOT, pharmacological management was suboptimal (zero to two antihypertensive drugs) in 62% of patients, and nutritional guideline adherence was suboptimal in 100% of patients (only 8% had a sodium intake on target, 66% had a potassium intake on target, 3% had a sodium-to-potassium ratio on target, and body mass index was <30 $kg/m^2$ in 35%). These data show pharmacological undertreatment and a low adherence to nutritional guidelines. Uncontrolled BP is common in T2DM, and our data show a window of opportunity for improving BP control, especially in nutritional management. To improve treatment quality, we advocate to incorporate the integrated monitoring of nutritional management in quality improvement cycles in routine care.

# Introduction

Type 2 Diabetes Mellitus (T2DM), with an estimated number of 422 million patients worldwide, is one of the major conditions associated with cardiovascular events and cardiovascular death [1]. Therefore, the prevention of the development and progression of such complications is a main goal in the treatment of T2DM, and evidence-based recommendations to reach this goal are incorporated in treatment guidelines. Treatment consists of pharmacological and non-pharmacological management, the latter consisting in large part of nutritional guidance. Still, cardiovascular complications develop in the majority of T2DM patients, demonstrating the large challenge of adequate treatment [2,3]. One explanation for this could be a failure to reach guideline treatment targets. Indeed, several studies have shown that targets for blood pressure, glycaemic control, and low density lipoprotein (LDL)cholesterol are not reached in a large number of patients [4–8].

Pharmacological and nutritional management are often studied as separate entities, despite the fact that both are crucial elements of treatment. We hypothesize that a systematic assessment of both pharmacological and nutritional management can identify targets for the improvement of treatment quality. The DIAbetes and LifEstyle Cohort Twente (DIALECT) cohort study was specifically designed for this purpose. DIALECT is an observational study in T2DM patients in a well-defined region in The Netherlands, and uses validated and detailed real-world data on nutritional habits, pharmacological treatment, and current clinical condition. To obtain non-biased data on individual nutrient intake, 24-hour urine collections were used and stored in a biobank to allow for future analyses [9].

We aim to address how well the targets for blood pressure management are reached, and how this is related to (1) pharmacological management, and (2) nutritional management (i.e., the dietary intake of salt [10,11], potassium [12,13], body mass index (BMI), and alcohol). Moreover, we assessed additional nutritional parameters for which no specific counselling was given, but have been shown to be relevant to cardiovascular risk in diabetic kidney disease (magnesium [14–16] and phosphate [17,18]). Because the presence of diabetic kidney disease implicates different blood pressure targets, we analysed patients without and with renal involvement separately.

## Materials and methods

*Study Design and Participants*

DIALECT is a prospective cohort study in patients with T2DM, performed in the ZGT Hospital, which is located in Almelo and Hengelo, The Netherlands. It is designed to study pharmacological and non-pharmacological management in a regional T2DM population treated in a secondary health care center. All patients with T2DM and aged 18+ years treated in the outpatient clinic of our hospital were eligible, with the only exclusion criteria being an inability to understand the informed consent procedure, insufficient knowledge of the Dutch language, or a dependency on renal replacement therapy.

This paper reports on the DIALECT-1 population, consisting of the first 450 patients, recruited between September 2009 and January 2016. The inclusion of new patients in DIALECT-2 will be performed until December 2019, or until the number of 850 is reached. The study is performed according to the guidelines of good clinical practice and the Declaration of Helsinki. It has been approved by the local institutional review boards (METC-registration numbers NL57219.044.16 and 1009.68020) and is registered in the Netherlands Trial Register (NTR trial code 5855).

*Study Procedures*

Patients were screened for eligibility in the electronic patient file, and subsequently invited for a study visit. At the clinic, all of the information relevant to the medical condition was recorded in a database (Figure 1, supplementary Table 1). Height, weight, and waist and hip circumference were measured. Body mass index was calculated as weight divided by height squared ($kg/m^2$), and body surface area was estimated by applying the universally adopted formula of DuBois [19]. Blood pressure was measured in a supine position by an automated device (Dinamap®; GE Medical systems, Milwaukee, WI, USA) for 15 minutes with a one-minute interval. The mean systolic and diastolic pressure of the last three measurements was used for further analysis.

Physical activity was assessed using the Short Questionnaire to Asses Health enhancing physical activity (SQUASH) questionnaire, which was previously validated in [20]. The 24-hour urinary content of specific substances was measured where possible and appropriate.

Routine laboratory tests were performed in venous blood, including blood count tests, liver function tests, renal function tests, HbA1c, and cholesterol. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [21]. From samples of a 24-hour urine collection, the

following parameters were measured: sodium, potassium, creatinine, calcium, phosphate, chloride, albumin, protein, urea, and uric acid excretion. Twenty-four-hour urinary excretion was calculated by multiplying these concentrations with the volume of the 24-hour urine collection. Creatinine clearance was calculated from the 24-hour urine creatinine excretion and the plasma creatinine concentration. For the proper collection of the 24-hour urine sample, patients were instructed to dispose of the first morning void urine, and thereafter collect all urine in the provided canister until the first morning void urine of the next day. In between voids, they were instructed to store the canister in a dark cool place, preferably in a refrigerator. A separate single morning void urine was used to assess the urinary albumin-to-creatinine ratio.

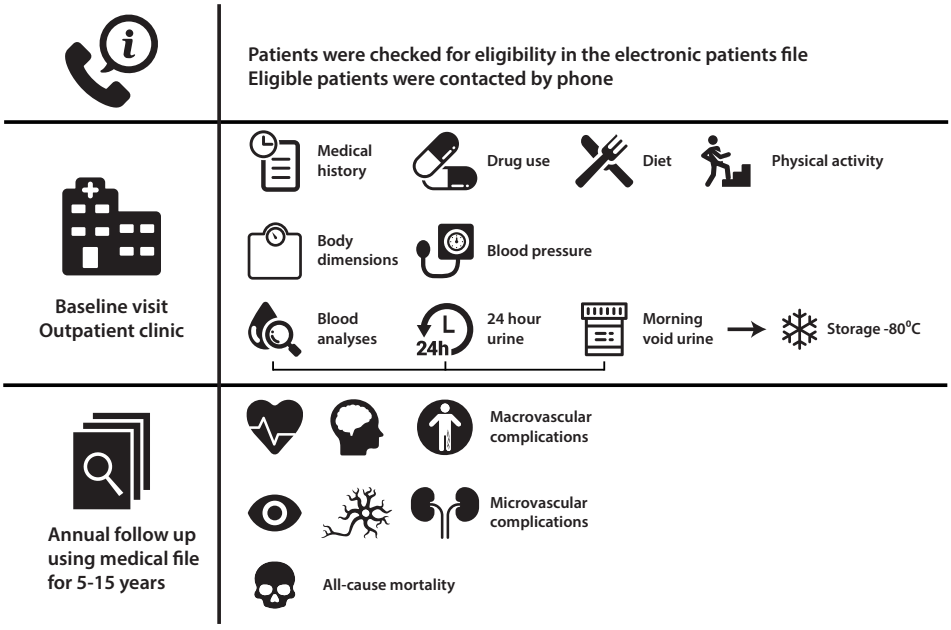


**Figure 1.** Patient inclusion and data collection in DIALECT.

The samples of blood, 24-hour urine collection, and morning void urine were stored in a biobank at–80 degrees Celsius for additional analyses, as specified in supplementary Table 1.

*Routine Clinical Care*

Diabetes care in the Netherlands is standardised, both in the outpatient clinic and at the general practitioner. It consists of three to four outpatient clinic visits per year. The development of albuminuria is assessed yearly using the albumin–creatinine ratio in a single

morning void urine. Retinopathy is assessed at one to two-year intervals. Neuropathy is assessed yearly using monofilament and vibration tests with a tuning fork.

Lifestyle management in T2DM consists of guidance regarding weight loss, increasing physical activity, and smoking cessation, and of referral to a dietician for dietary guidance on weight loss and the adherence to dietary guidelines, including sodium restriction and stimulating an intake of fruit and vegetables. The frequency of dietary follow-up visits is targeted at the individual goals and needs of patients depending on personal preferences as well as comorbidity. At each doctor visit, target HbA1c and blood pressure are monitored, and pharmacological intervention is adjusted accordingly. Cholesterol levels are monitored yearly. Targets for HbA1c and LDL cholesterol are often individualized; the general targets are <53 mmol/L and <2.5 mmol/L, respectively.

*Definitions*

The blood pressure (BP) targets in our analyses were derived from the international guidelines for diabetes management, which have been adopted for use in The Netherlands [22,23]. In patients with diabetic kidney disease, the BP target was set according to the Kidney Diseases Improving Global Outcomes (KDIGO) guidelines, which are internationally acclaimed guidelines for chronic kidney disease, and are also applied in The Netherlands [23]. Patients with diabetic kidney disease without albuminuria (eGFR <60, no albuminuria) had a BP target of ≤140/90 mmHg, while patients with albuminuria and either an eGFR ≥60 ml/min•1.73m$^2$ or an eGFR <60 ml/min•1.73m$^2$ had a BP target of ≤130/80 mmHg. For patients with T2DM without diabetic kidney disease, the European Association for the Study of Diabetes (EASD) guidelines are used, which stipulate a blood pressure (BP) target of <140/85 mmHg [22]. Accordingly, the patients were grouped by eGFR above or below 60 ml/min•1.73m$^2$ and by the presence of albuminuria. Albuminuria was defined as a 24-hour urinary albumin excretion >30 mg/day. As the EASD and KDIGO guidelines for those without albuminuria differ slightly, we performed all of the analyses using the EASD guidelines for those with eGFR <60 and no albuminuria as well. The results were virtually similar, and for the sake of conciseness, the data is not shown. The targets for nutritional management were set according to the Dutch guidelines when available. The target dietary salt intake was ≤6 g/day [24], and the target dietary potassium intake was set at ≥3.5 g/day, according to best evidence [13]. The target alcohol intake was ≤2 units per day for women, and ≤3 units per day for men. It should be noted that in 2015, the Health Council of The Netherlands changed the guidelines for alcohol consumption to zero units per day; however, our patients were included in the study before the introduction of these new guidelines [25]. The target BMI was <30 kg/m$^2$. The target for smoking was either no smoking history or having previously stopped smoking.

The data on dietary intake of salt, potassium, and proteins were derived from 24-hour urinary excretion. For this, it is important to realise that the patients in our cohort were assessed under steady state conditions, in which the net renal excretion of sodium is almost equal to the dietary intake of sodium, with only approximately 5–10% being excreted by other routes (e.g., sweat or feces) [9]. Therefore, 24-hour urinary sodium excretion is considered the gold standard for the assessment of sodium intake [9,26], and dietary salt intake was calculated by multiplying the net 24-hour sodium excretion (in mol/day) with the molar weight of salt (NaCl, 58.44 g/mol). Dietary potassium intake was calculated from urinary potassium excretion under the assumption of a renal excretion rate of 77% [13,27]. Dietary protein intake was calculated from urinary urea nitrogen excretion using the Maroni formula [28]. As the renal excretion of magnesium is lower in patients with a low eGFR, dietary magnesium intake could not be calculated from urinary magnesium excretion with the same formula (using the assumption of an intestinal absorption of 30%) [16]. Therefore, we present the urinary daily excretion of magnesium. Also, while no consensus exists to calculate dietary phosphate intake from the urinary excretion, urinary phosphate excretion does reflect variability in intestinal phosphate uptake [29,30], so we present the urinary excretion values.

*Statistical Analyses*

All of the statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 23.0. Normally distributed data are presented as mean ± standard deviation. Skewed variables are expressed as median [interquartile range]. Dichotomous variables are presented in number and percentage. First, we divided the population according to the presence of albuminuria and/or a reduced eGFR (<60 ml/min•1.73m$^2$), as in these groups the target BP is different (<140/85 for those without diabetic kidney disease, ≤140/90 mmHg for patients without albuminuria and an eGFR <60 ml/min•1.73m$^2$, and ≤130/80 mmHg for those with albuminuria). Second, we divided the population into two groups, according to the reached blood pressure. These groups are denoted as "Blood pressure on target" (BP-OT) and "Blood pressure not on target" (BP-NOT), respectively. The differences between the groups were analysed using the student t-test, one-way ANOVA, the Mann–Whitney U test, the Kruskal–Wallis test, and the Chi Square test when appropriate. To perform a multivariate analysis of the determinants of not on target BP, multivariate logistic regression was used. In order to adjust for age and gender, the differences in nutritional data among the groups were also determined using mixed model analyses with Sidak post-hoc tests.

# Results

Between September 2009 and January 2016, 1082 eligible patients were identified and invited to participate in the study, of whom 470 were enrolled in the study and performed the baseline visit. The most common causes for not participating in the study were: No interest in research, and inability due to co-morbidity (Figure 2). Twenty patients were excluded after the baseline visit, as in closer analysis their correct diagnosis was Type 1 Diabetes Mellitus instead of Type 2. All of the remaining 450 patients were included in our data analysis.

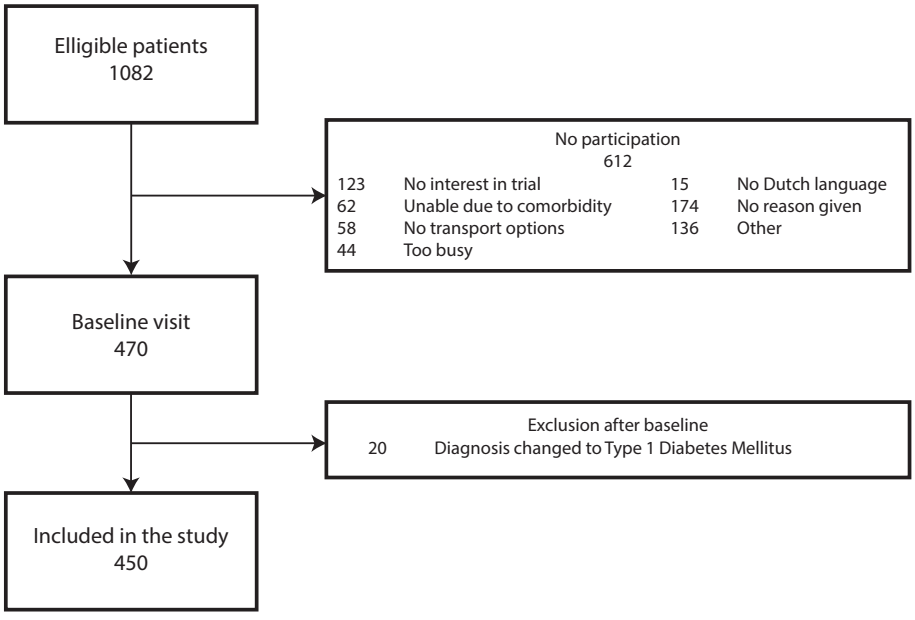


**Figure 2.** Patient recruitment flowchart.

*Baseline Pharmacological and Nutritional Characteristics*

The baseline data are presented in Table 1, by a break-up according to reduced eGFR (<60 ml/min•1.73m$^2$) and the presence of albuminuria. The mean age of the participants was 63 ± 9 years, and was higher in the groups with eGFR <60 (Table 1). There were more men (58%) than women, and men were over-represented in the albuminuria groups (74% and 77% respectively for eGFR ≥60 and <60). The mean BMI was 32.9 ± 6.2 kg/m$^2$, reflecting a predominantly obese T2DM population, and BMI did not differ among the groups (Table 1).

There was no renal involvement in 57% of the patients (eGFR ≥60/Alb–; Table 1). Of all of the patients, 30% (*n*=136) had albuminuria, either with a preserved (*n*=85, eGFR ≥60/Alb+) or reduced renal function (*n*=51, eGFR <60/Alb+). Fifty-two patients (12%) had a reduced renal function without albuminuria (eGFR <60/Alb–). The mean systolic blood pressure was 139 ± 16 mmHg, and the mean diastolic BP was 76 ± 9 mmHg. Most of the patients (81%) used one or more antihypertensive drugs. The target BP was reached in 53% of all patients, while 47% had BP not on target. In patients with albuminuria, 33% and 24% reached the target blood pressure in eGFR ≥60 and in eGFR <60, respectively (Table 1). Additionally, a blood pressure of ≤140/90 mmHg was reached in 48% and 41% of albuminuria patients with an eGFR ≥60 and eGFR <60, respectively. The group with albuminuria and eGFR <60 received the largest number of antihypertensive drugs (3 [2-4] drugs, Table 1). Additionally, the number of patients with hypertension requiring 4+ drugs was highest in this group (59%, P <0.001). In contrast, the antihypertensive drug use in the eGFR ≥60/Alb+ group is not higher than in the other groups (2 [1-3] drugs). Patients without chronic kidney disease (CKD) (Table 1, group eGFR ≥60/Alb–) most commonly used renin-angiotensin-aldosterone-system inhibition (RAASi) (59%), followed by β-blockers (39%), and thiazide diuretics (32%). This was different in those with CKD (groups eGFR ≥60/Alb+, eGFR <60/Alb–, and eGFR <60/Alb+): RAASi (77%), β-blockers (62%), and Calcium antagonists (31%). There were two patients with an eGFR <60 that used a phosphate binder, one in the Alb– group, and one in the Alb+ group.

The mean dietary salt intake was high, namely, 10.9 g of salt per day, and was considerably higher in the groups with preserved eGFR. When adjusting for age and gender, these differences remained virtually similar (data not shown). In the overall population, only 53 patients (12%) adhered to the dietary guidelines for dietary salt intake, ≤6 g/day, and in the eGFR ≥60/Alb+ group this percentage was even lower, i.e., 6%. In total, 8% of patients had a salt intake of ≤5 g/day as recommended by the WHO. The mean potassium intake was 3.9 ± 1.3 g/day, and 66% of patients had an intake, as recommended, above 3.5 g/day (Table 1). The mean urinary magnesium excretion was 4.0 ± 2.1 mmol/day, and as expected was lower in patients with an eGFR <60 ml/min•1.73m$^2$ than in those with an eGFR ≥60 ml/min•1.73m$^2$. The mean urinary phosphate excretion was 27.5 ± 11.6 mmol/day, and the mean calculated dietary protein intake was 92 ± 27 g/day.

**Table 1.** DIALECT-1 Baseline, nutritional, and pharmacological characteristics

| Variable | Total Population | eGFR ≥60 Albuminuria No |
|---|---|---|
| Number of patients (% of population) | 450 | 257 (57) |
| ***Patient characteristics*** | | |
| Age (years) | 63 ± 9 | 61 ± 9 |
| Male, *n* (%) | 259 (58) | 139 (54) |
| Years T2DM (years) | 11 [7-18] | 11 [7-18] |
| Serum HbA1C (mmol/mol) | 57 ± 12 | 58 ± 11 |
| Insulin use, *n* (%) | 284 (63) | 160 (62) |
| Systolic blood pressure (mmHg) | 139 ± 16 | 136 ± 15 |
| Diastolic blood pressure (mmHg) | 76 ± 9 | 75 ± 9 |
| BP on target, *n* (%) | 236 (53) | 155 (60) |
| Macrovascular disease, *n* (%) | 158 (35) | 68 (27) |
| eGFR (ml/min•1.73m$^2$) | 84 [62-97] | 92 [78-100] |
| Albumin excretion (mg/day) | 11 [3-66] | 5 [2-11] |
| ***Pharmacological management*** | | |
| RAASi, *n* (%) | 296 (67) | 152 (59) |
| β-blockers, *n* (%) | 207 (46) | 100 (39) |
| Thiazide diuretics, *n* (%) | 137 (31) | 81 (32) |
| Calcium antagonists, *n* (%) | 101 (23) | 43 (17) |
| Loop diuretics, *n* (%) | 81 (18) | 26 (10) |
| Potassium sparing diuretics, *n* (%) | 43 (10) | 11 (4) |
| Number of antihypertensives | 2 [1-3] | 2 [0-3] |
| No antihypertensive therapy, *n* (%) | 83 (19) | 65 (25) |
| 1 drug, *n* (%) | 101 (23) | 61 (24) |
| 2 drugs, *n* (%) | 106 (24) | 57 (22) |
| 3 drugs, *n* (%) | 91 (20) | 44 (17) |
| 4 drugs, *n* (%) | 56 (13) | 24 (9) |
| 5+ drugs, *n* (%) | 11 (3) | 6 (2) |
| Hypertension requiring 4+ drugs, *n* (%) | 117 (26) | 48 (19) |
| Total number of drugs | 7 ± 3 | 6 ± 3 |
| ***Non-pharmacological management*** | | |
| BMI (kg/m$^2$) | 32.9 ± 6.2 | 32.9 ± 6.5 |
| Current smoker, *n* (%) | 74 (17) | 41 (16) |
| Alcohol intake (units per month) | 5 [0-30] | 5 [0-28] |
| 25(OH) Vitamin D (nmol/L) | 42 ± 20 | 43 ± 18 |
| *Urinary excretion* | | |
| Urinary creatinine excretion (mmol/day) | 13.8 ± 4.8 | 13.9 ± 4.9 |
| Urinary magnesium excretion (mmol/day) | 4.0 ± 2.1 | 4.1 ± 2.1 |
| Urinary phosphate excretion (mmol/day) | 27.5 ± 11.6 | 28.2 ± 12.2 |
| Sodium-to-potassium ratio (mmol/mmol) | 2.5 ± 1.0 | 2.5 ± 1.0 |
| *Calculated intake* | | |
| Dietary salt intake (g/day) | 10.9 ± 4.7 | 11.0 ± 4.3 |
| Salt intake ≤6 g/day | 53 (12) | 26 (10) |
| Dietary potassium intake (g/day) | 3.9 ± 1.3 | 4.0 ± 1.4 |
| Potassium intake ≥3.5 g/day | 290 (66) | 173 (69) |
| Dietary protein intake (g/day) | 92 ± 27 | 94 ± 28 |

* P <0.05 vs. eGFR ≥60/Albuminuria (Alb)–; † P <0.05 vs. eGFR ≥60/Alb+. ‡ P <0.05 vs. eGFR <60/Alb–. T2DM, Type 2 Diabetes Mellitus; BP, blood pressure.

| | eGFR ≥60 | | eGFR <60 | |
|---|---|---|---|---|
| | Albuminuria Yes | Albuminuria No | Albuminuria Yes | *P*-Value |
| | 85 (19) | 52 (12) | 51 (11) | |
| | | | | |
| | 62 ± 8 | 67 ± 8 *,† | 69 ± 7 *,† | <0.001 |
| | 63 (74) | 19 (37) | 39 (77) | <0.001 |
| | 14 [8-19] | 12 [6-17] | 10 [6-15] | 0.45 |
| | 59 ± 13 | 54 ± 11 | 57 ± 13 | 0.15 |
| | 64 (75) | 31 (60) | 28 (55) | 0.07 |
| | 140 ± 19 | 131 ± 13 † | 139 ± 17 | 0.009 |
| | 76 ± 10 | 70 ± 9 *,† | 75 ± 10 ‡ | 0.004 |
| | 28 (33) | 41 (79) | 12 (24) | <0.001 |
| | 36 (42) | 25 (48) | 31 (61) | <0.001 |
| | 88 [74-99] | 47 [36-54] | 39 [33-45] | <0.001 |
| | 94 [62-202] | 4 [1-12] | 332 [93-661] | <0.001 |
| | | | | |
| | 63 (74) | 39 (75) | 42 (82) | 0.001 |
| | 37 (44) | 36 (69) | 33 (65) | <0.001 |
| | 15 (18) | 21 (40) | 18 (35) | 0.02 |
| | 26 (31) | 13 (25) | 19 (37) | 0.002 |
| | 18 (21) | 17 (33) | 20 (39) | <0.001 |
| | 8 (9) | 12 (23) | 12 (24) | <0.001 |
| | 2 [1-3] | 3 [2-3] | 3 [2-4] | <0.001 |
| | 12 (14) | 1 (2) | 2 (4) | <0.001 |
| | 17 (20) | 6 (12) | 6 (12) | |
| | 28 (33) | 13 (25) | 11 (22) | |
| | 15 (18) | 21 (40) | 12 (24) | |
| | 10 (12) | 8 (15) | 13 (26) | |
| | 3 (4) | 3 (6) | 7 (14) | |
| | 23 (27) | 16 (31) | 30 (59) | <0.001 |
| | 7 ± 2 | 8 ± 3 * | 9 ± 3 *,† | <0.001 |
| | | | | |
| | 32.9 ± 5.4 | 33.3 ± 6.2 | 32.3 ± 6.1 | 0.89 |
| | 15 (18) | 10 (19) | 8 (16) | 0.93 |
| | 10 [0-47] | 3 [0-24] | 12 [0-40] | 0.22 |
| | 37 ± 19 | 42 ± 26 | 44 ± 22 | 0.09 |
| | | | | |
| | 14.8 ± 5.4 | 12.8 ± 4.2 | 12.8 ± 3.6 | 0.03 |
| | 4.4 ± 2.3 | 3.3 ± 1.7 *,† | 3.2 ± 1.4 *,† | 0.001 |
| | 30.3 ± 12.6 | 22.7 ± 7.7 *,† | 25.0 ± 7.9 | 0.001 |
| | 2.8 ± 1.2 | 2.2 ± 0.7 † | 2.3 ± 0.8 | 0.004 |
| | | | | |
| | 12.7 ± 5.6 * | 8.7 ± 4.0 *,† | 9.7 ± 3.9 † | <0.001 |
| | 5 (6) | 15 (29) | 7 (14) | <0.001 |
| | 4.1 ± 1.1 | 3.5 ± 1.3 | 3.6 ± 0.9 | 0.01 |
| | 62 (73) | 27 (53) | 29 (59) | 0.06 |
| | 98 ± 29 | 80 ± 23 *,† | 84 ± 21 † | 0.001 |

*Pharmacological and Nutritional Management in BP-On Target (BP-OT) and BP-Not On Target (BP-NOT) Groups*

Table 2 shows the patients' characteristics by a break-up of BT-OT and BP-NOT. Patients with BP-NOT were more often men (64% vs. 53%, P=0.018), and had a higher HbA1C (59 ± 12 vs. 56 ± 11 mmol/L, P=0.031). While the presence of albuminuria was a strong predictor of uncontrolled BP (46% vs. 17%, P <0.001), poor BP control was not associated with an eGFR <60 ml/min•1.73m$^2$ (24% vs. 22%).

Patients with BP-OT used loop diuretics more often than those with BP-NOT (Table 2). There were no other differences in the pharmacological treatment between the BP groups; neither in the types of prescribed drugs, nor in the total number of prescribed antihypertensive drugs. Surprisingly, of the patients with BP-NOT, 21% did not use any antihypertensive drug, while 20% used only one, and 21% used two antihypertensive drugs. Adherence to the recommended nutritional guidelines by a breakup of BP-OT and BP-NOT is shown in Figure 3. In both groups, adherence to the recommended lifestyle guidelines was poor, and the total number of lifestyle targets adhered to did not differ between the groups (3 [2-3] in BP-OT vs. 3 [2-3] in BP-NOT, P=0.22). In patients with BP-NOT, 8% had a dietary salt intake below the recommended 6 g/day, which was lower than those with BP-OT (15%,=0.025). Adherence to the potassium guideline (66% of patients) did not differ among the groups. Only 3% of patients had a sodium-to-potassium ratio ≤1.0 in both BP groups. There were only three patients (1%) who adhered to both the recommended intakes of salt and potassium. BMI was ≤30 kg/m$^2$ in 35% of patients, and this proportion did not differ among the BP groups. The smoking and alcohol guidelines were adhered to by 83% and 86% of all patients, and these proportions were not different among the BP groups. We found no differences in the other nutritional factors between the BP-OT and BP-NOT groups (Table 2). In the total population, there was only one patient (with BP-OT) who adhered to all of the lifestyle guidelines simultaneously. There were no differences in lifestyle guidelines adherence between those with zero to two antihypertensives and those with three-plus antihypertensives.

In the multivariate logistic regression analysis, albuminuria and the use of loop diuretics remained the only significant predictors of BP-NOT (data not shown).

**Table 2.** DIALECT-1 pharmacological and nutritional management by a breakup of BP on target/not on target

| Variable | BP On Target | BP Not On Target | *P*-Value |
|---|---|---|---|
| *Patient characteristics* | *n*=239 | *n*=210 | |
| Age (years) | 63 ± 9 | 63 ± 9 | 0.36 |
| Male, *n* (%) | 126 (53) | 134 (64) | 0.02 |
| Years T2DM (years) | 11 [7-17] | 12 [7-18] | 0.26 |
| Serum HbA1C (mmol/mol) | 56 ± 11 | 59 ± 12 | 0.03 |
| Insulin use, *n* (%) | 149 (62) | 136 (65) | 0.60 |
| Systolic blood pressure (mmHg) | 125 ± 10 | 149 ± 13 | <0.001 |
| Diastolic blood pressure (mmHg) | 70 ± 8 | 80 ± 9 | <0.001 |
| eGFR < 60, *n* (%) | 53 (22) | 51 (24) | 0.60 |
| Albuminuria, *n* (%) | 40 (17) | 95 (46) | <0.001 |
| *Pharmacological management* | | | |
| RAASi, *n* (%) | 163 (68) | 134 (64) | 0.33 |
| β-blockers, *n* (%) | 115 (48) | 93 (44) | 0.42 |
| Thiazide diuretics, *n* (%) | 71 (30) | 66 (31) | 0.69 |
| Calcium antagonists, *n* (%) | 50 (21) | 52 (25) | 0.33 |
| Loop diuretics, *n* (%) | 52 (22) | 29 (14) | 0.03 |
| Potassium sparing diuretics, *n* (%) | 22 (9) | 21 (10) | 0.78 |
| Number of antihypertensives | 2 [1-3] | 2 [1-3] | 0.51 |
| No antihypertensive therapy, *n* (%) | 39 (16) | 44 (21) | 0.85 |
| 1 drug, *n* (%) | 47 (20) | 42 (20) | |
| 2 drugs, *n* (%) | 64 (27) | 45 (21) | |
| 3 drugs, *n* (%) | 50 (21) | 43 (21) | |
| 4 drugs, *n* (%) | 29 (12) | 27 (13) | |
| 5+ drugs, *n* (%) | 10 (4) | 9 (4) | |
| Hypertension requiring 4+ drugs, *n* (%) | 39 (16) | 79 (38) | <0.001 |
| Total number of drugs | 7.0 ± 2.6 | 6.7 ± 2.8 | 0.30 |
| *Non-pharmacological management* | | | |
| BMI (kg/m$^2$) | 32.8 ± 5.8 | 32.9 ± 6.7 | 0.89 |
| Serum 25 (OH) Vitamin D (nmol/L) | 43 ± 20 | 41 ± 20 | 0.22 |
| *Urinary excretion* | | | |
| Urinary creatinine excretion (mmol/day) | 13.6 ± 4.9 | 14.1 ± 4.7 | 0.22 |
| Urinary magnesium excretion (mmol/day) | 3.9 ± 2.1 | 4.0 ± 1.9 | 0.43 |
| Urinary phosphate excretion (mmol/day) | 26.9 ± 12.3 | 28.2 ± 10.7 | 0.26 |
| Sodium-to-potassium ratio (mmol/mmol) | 2.5 ± 1.0 | 2.5 ± 0.9 | 0.49 |
| *Calculated intake* | | | |
| Dietary salt intake (g/day) | 10.7 ± 4.8 | 11.1 ± 4.4 | 0.47 |
| Dietary potassium intake (g/day) | 3.8 ± 1.3 | 4.0 ± 1.2 | 0.15 |
| Dietary protein intake (g/day) | 90 ± 29 | 93 ± 26 | 0.29 |

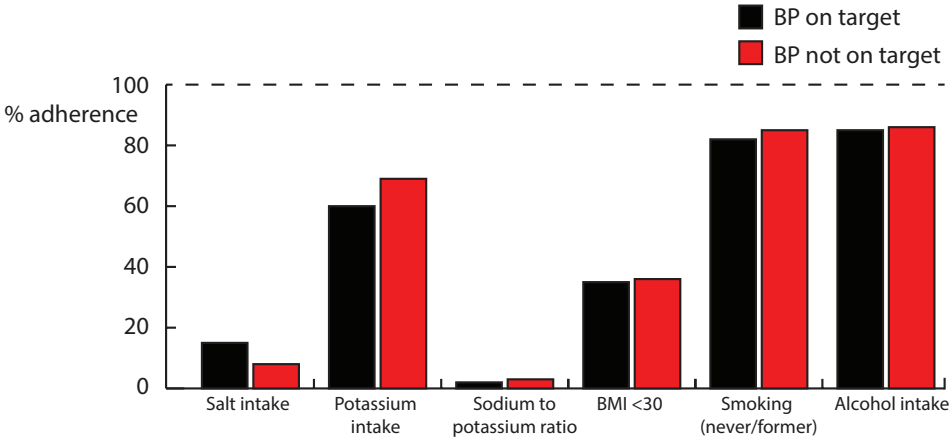


**Figure 3.** % Adherence to nutritional guidelines, by a breakup of blood pressure on target/not on target. Alcohol intake is self-reported, and the target intake was ≤2 units per day for women and ≤3 units per day for men.

## Discussion

In this paper, we present the blood pressure management of Type 2 diabetes patients (T2DM), using combined data on pharmacological and nutritional management in a real-life secondary health care setting. As anticipated, the prevalence of hypertension was high, and 81% of patients were on antihypertensives. However, BP control was poor, as the target BP was not reached in 47% of patients. An integrated assessment of pharmacological and nutritional management demonstrated a large window of opportunity for improving BP control in T2DM, both by intensifying antihypertensive drug treatment, and increasing nutritional guideline adherence.

The proportion of patients with their blood pressure on target (BP-OT) in our cohort is in line with findings in other T2DM cohorts [31], as well as diabetic kidney disease cohorts [5,32]. The proportion was lower than found in T2DM patients treated in the primary care setting, in whom adequate blood pressure control was found in 85% of patients [33,34], which may well reflect the referral policy, with more difficult patients being referred to secondary care. In the baseline data of the LEADER-4 trial (a randomized clinical trial in T2DM patients) [31], where 51% of patients had BP-NOT, the antihypertensive use was lower than we report, as about 80% of all patients used zero to two antihypertensive drugs. In diabetic kidney disease studies (i.e., studies with T2DM and either albuminuria and/or eGFR <60), the antihypertensive drug use is mostly in line with our findings, with >90% of patients using antihypertensive drugs, and RAASi being the most frequently used drug [5,32]. In line with our findings, Smits *et al.* found that RAASi was the most commonly used class of antihypertensive drugs in a Dutch T2DM cohort in primary care, followed by betablockers and diuretics [33]. The number of used antihypertensive drugs they report is largely comparable to our findings, albeit that the use of four to five drugs seems more common in our secondary care cohort. It should be noted that in these studies regarding BP control in a real-world setting, data regarding nutrient intake is not available.

An important issue when evaluating the pharmacological management of blood pressure is treatment non-adherence, which reportedly ranges from 31 to 40% in patients with poorly controlled blood pressure[35,36]. Thus, establishing an infrastructure that allows the monitoring of adherence would be of great value. Yet, even assuming a drug-treatment non-adherence rate of 40% in our patients, the non-adherence to lifestyle measures seems to stand out as an additional important target for intervention.

What can be done to improve BP control in T2DM? Our data, and data from other trials, clearly show that true therapy-resistant hypertension, defined as hypertension persisting despite three antihypertensive drugs at maximum tolerable dosage of which one is a

diuretic, is not the issue in most patients with BP-NOT. The majority of BP-NOT patients (62%) do not use more than two antihypertensive drugs, illustrating the opportunity for intensifying pharmacological treatment. One promising option in this regard is the removal of excess extracellular fluid with diuretics, especially in those patients with a high salt intake.

Our data show that, especially for nutritional management, there is a large window of opportunity for improvement, as in the total population only one patient adhered to all of the nutritional guidelines simultaneously. This is highly relevant, since lifestyle interventions have the potential to not only reduce BP, but to also reduce the overall cardiovascular risk [11,37–40]. Even though dietary counselling has already been part of their routine care, the mean daily salt intake in our population was almost 11 g/day, roughly twice that of the recommended 6 g/day, and considerably higher than the mean salt intake in the general Dutch population of 8.5 g/day [41]. Previously, Mente *et al* demonstrated that for each 1-gram increment in estimated sodium excretion, blood pressure was 2/1 mmHg higher, where this slope was more pronounced and steeper in those with hypertension, high-sodium diets (>5g/day), and older persons [42]. Therefore, the most obvious step to improve non-pharmacological management would be to reduce dietary salt intake. This is underscored by a previous study, performed in T2DM patients in the same region, which has shown that, although the aim to reduce dietary salt intake to <6 g/day was not reached, even a relatively modest reduction in salt intake from 12 to 9 g/day can reduce blood pressure by 6/3 mmHg and albuminuria by 42% while under RAASi [43]. Furthermore, reducing salt intake is associated with potentiating the antihypertensive effects of RAASi [44–46].

There is evidence that a combined dietary approach aimed at reducing salt while increasing potassium intake has the potential to improve cardiovascular risk management [12,47]. However, the potassium intake in our patients was generally already above the recommended intake of 3.5 g/day. Therefore, the finding that the sodium-to-potassium ratio was higher than the deemed optimal ratio of 1 mmol/mmol in 97% of patients is primarily determined by high salt intake.

To improve blood pressure control, dietary intervention could also be aimed at reducing body weight. The mean BMI in our cohort was above 30 kg/m$^2$. While a relationship between obesity and blood pressure has previously been demonstrated [7,48], we did not find such an association here. This might be due to the fact that we had few participants with a BMI <25 kg/m$^2$, and therefore did not have a large enough dispersion to differentiate between the BP groups. Intentional weight loss has been associated with beneficial effects, both on BP and on other cardiovascular risk factors such as LDL cholesterol and glycaemic control [49]. Therefore, even though weight loss is notoriously difficult to

achieve, especially in patients on insulin treatment, it should remain a priority in the non-pharmacological treatment of T2DM, and also in secondary health care centres.

Finally, an association between the intake of magnesium and phosphate and blood pressure has been reported previously [14,17,18,50,51]. Here, we did not find differences in urinary magnesium excretion or in urinary phosphate excretion between those with BP-OT and BP-NOT. As the urinary excretion of magnesium and phosphate is lower in those with a low eGFR, these results might be misleading. However, the proportion of patients with a low eGFR was similar in both the BP-OT and BP-NOT groups, making it less likely that differences in the urinary excretion of magnesium and phosphate between the BP groups were masked by differences in urinary excretion due to a low eGFR. In the general population, a continuous relationship between lower magnesium excretion and the risk for hypertension was reported [16]; moreover, patients with a low magnesium intake had a greater risk of developing ischemic heart disease [52]. While no nutritional recommendations are currently available for magnesium to stratify adequate/inadequate intake, in our population approximately 28% of patients had a magnesium excretion below the values associated with ischemic heart disease in the general population. Regarding phosphate intake, population-based studies as well as studies in CKD have shown associations with outcome, albeit not equivocal [53,54], and it has been proposed that excess phosphate intake is a risk factor that is generally overlooked in patients with early stages of CKD by lack of measurements [55]. While more research is needed on the relation between magnesium and phosphate excretion and adverse outcomes, our data illustrate that 24-hour urine, collected to assess the intake of established nutritional targets such as salt and potassium, can simply be used to establish a more complete nutritional profile, which could be useful for future improvements in nutritional studies and counselling.

It should be noted that the adherence to nutritional guidelines was equally poor in the BP-OT and BP-NOT groups. While in the BP-NOT group there is more urgency to adhere to these guidelines, namely to correct BP, the adherence to the guidelines in the BP-OT group should not be overlooked. In regard to salt intake, previously it has been shown that a higher salt intake while under RAASi is associated with worse cardiovascular outcomes, even independent of BP [46]. Furthermore, as stated above, intentional weight loss has many benefits that surpass BP management [49], and therefore can also greatly improve outcomes if BP is already on target. Lastly, in a population-based cohort, low potassium intake has been associated with the occurrence of chronic kidney disease [56].

The DIALECT study has several strengths, including the use of real-world data from a cohort representative of secondary health care in T2DM, at least in the context of the Dutch referral health care setting. Second, we study the integrated role of non-bi-

ased data on both pharmacological and non-pharmacological parameters on BP, which is an important approach, as in cardiovascular risk management pharmacological and non-pharmacological interventions go hand in hand. Third, through the use of 24-hour urine collections, we provide objective measurements of dietary intake and several relevant nutrients. There are also some limitations. An observational study cannot prove causal relationships. Also, there is some risk of response bias, although patient characteristics were similar between those who did and did not participate.

What are the implications of our study? Adequate management equals the sum of measures taken in combination with compliance. Our data on poor nutritional management do not distinguish between a lack of adequate nutritional counselling and a lack of compliance. However, it is well established that sustained lifestyle change is difficult to achieve, demonstrating that currently no modus of adequate counselling and therefore adequate management exists. The question, therefore, is how to establish this. Previous well-designed studies, using interventions of intensive nurse practitioner support and self-management, both did not lead to neither long-standing changes in nutritional habits, nor a reduction of cardiovascular outcomes [57,58]. As alternative approach, improvement strategies as tested for pharmacological management could be considered. In particular, it has been shown that the systematic evaluation of prescription quality as assessed by prescription quality indicators not only improved pharmacological compliance with guidelines, but also patient outcomes [59]. To the best of our knowledge, such approaches have never been developed and tested for nutritional management. As several objective parameters are available, such as the urinary excretion of sodium and potassium, this would be feasible in routine clinical care. Therefore, to improve blood pressure control, in our opinion, the use of nutritional quality indicators may have the potential to improve treatment quality as a whole.

## Conclusions

Uncontrolled BP is common in T2DM, especially in those with microalbuminuria. An integrated assessment of pharmacological and nutritional management demonstrated a window of opportunity for improving BP treatment, especially in nutritional management. We advocate that incorporating the integrated monitoring of pharmacological and nutritional management in quality control cycles has the potential to improve treatment quality in T2DM.

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**Supplementary Table 1.** Data collection in DIALECT-1

| |
|---|
| **Medical history** |
| Medical history interview, review of electronic patient file |
| **Medication use** |
| Medication interview, drug overview from the pharmacist |
| **Questionnaires** |
| *General* |
| Smoking history, second hand smoking, occupation, nationality |
| *Physical activity* |
| Short QUestionnaire to ASses Health enhancing physical activity (SQUASH) |
| *Dietary habits* |
| Food-Frequency Questionnaire (FFQ) |
| **Blood pressure** |
| In a separate room, blood pressure is measured each minute during 15 minutes, while the patient is sitting in a supine position. |
| **Physical examination** |
| *General* |
| Height, weight, waist circumference, hip circumference |
| *Neuropathy* |
| Monofilament and VibraTip™ |
| *Body impedance* |
| Bodyscan® Quadscan 4000 and the TANITA® BC418MA |
| **Laboratory assessments** |
| *Serum* |
| Hemoglobin, hematocrit, erythrocyte sedimentation rate, leukocyte and platelet count, c-reactive protein, total cholesterol, LDL- and HDL cholesterol, triglycerides, HbA1c, glucose, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, albumin, total protein, N-terminal prohormone of brain natriuretic peptide, creatinin, eGFR (CKD-epi), urea, uric acid, sodium, potassium, calcium, phosphate, vitamin D, parathyroid hormone, magnesium, thyroid-stimulating hormone, free thyroxine, venous blood gas analysis, lactate |
| *24h urine* |
| Volume, creatinine excretion, sodium excretion, potassium excretion, calcium excretion, phosphate excretion, urea excretion, uric acid excretion, magnesium excretion, total protein excretion |
| *Morning void urine* |
| Dipstick test for erythrocytes, leucocytes, glucose, ketones, nitrite and pH |
| Albumine and albumine-to-creatinine ratio |
| **Biobanking** |
| *Serum:* 10x serum, 9x EDTA, 1x EDTA + glutathion, 6x citrated, 10x heparin, 1x whole blood |
| *24h urine collection:* 10x regular, 5x acidified to pH<2, 5x alcalized to pH>8 |
| *Single morning void collection:* 5x regular |

**Supplementary Table 1.** Continued

| **Follow up** |
|---|
| *Continuous data* |
| Blood pressure, Weight, Pharmacological treatment, HbA1c, LDL-cholesterol, eGFR, Urinary albumin-to-creatinine ratio |
| *Endpoints* |
| Macrovascular events |
| Microvascular events |
| Renal events |
| Mortality, all-cause and cardiovascular |

# CHAPTER 3

## Real-Life Achievement of Lipid-Lowering Treatment Targets in the DIAbetes and LifEstyle Cohort Twente: Systemic Assessment of Pharmacological and Nutritional Factors

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# Abstract

**Background/Objectives** Lowering low density lipoprotein cholesterol (LDLc) in type 2 diabetes mellitus is of paramount importance in preventing cardiovascular disease. However, treatment targets for LDLc are often not reached. We studied the prevalence of LDLc target achievement in a real-life population of type 2 diabetes mellitus patients in secondary care, and investigated whether in those not on target, there is room for intensifying pharmacological and lifestyle management according to current treatment guidelines.

**Subjects/Methods** We performed a cross-sectional analysis in the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1; *n*=450, age 63 ± 9 years, 58% men, diabetes duration 11 [7-18] years). At baseline, we determined plasma LDLc concentration, pharmacological treatment (i.e. statin use), and lifestyle (physical activity and dietary intake). Patients were divided according to LDLc <1.8, LDLc 1.8-2.5 and LDLc >2.5 mmol/l. Dietary intake was collected from a validated Food Frequency Questionnaire (177 items), and we determined guideline adherence for different food groups. Physical activity was assessed with the Short Questionnaire to ASsess Health enhancing behavior.

**Results** LDLc data were available in 428 type 2 diabetes mellitus patients. LDLc ≤2.5 mmol/l was achieved in 317 patients (76%). In total, 76% of patients used statins, in those with LDLc >2.5 mmol/l this was 44%. Adherence to lifestyle guidelines was not different between the LDLc groups, and was as follows: Body mass index 6%; physical activity 59%; vegetables 7%; fruit 28%; legumes 59%; nuts 14%; dairy 19%; fish 36%; tea 8%; fats 66%; red meat 12%; processed meat 2%; alcohol 71%; sweetened beverages 34%; sodium 12%.

**Conclusions** In type 2 diabetes mellitus patients in secondary health care, the target LDLc is achieved by three quarters of patients. Increasing statin treatment could be a first step to improve LDLc. Additionally, there are ample opportunities for lifestyle management through increasing adherence to lifestyle guidelines.

# Introduction

Type 2 diabetes mellitus is associated with a substantially increased risk for cardiovascular disease (CVD), of up to 2 times higher than the general population, especially if disease duration is >10 years[1,2]. Prevention of cardiovascular complications is therefore one of the main aims in the overall treatment for type 2 diabetes mellitus, with appropriate treatment of dyslipidemia as one of the major goals. Lowering of low density lipoprotein cholesterol (LDLc) in type 2 diabetes mellitus consistently reduces cardiovascular events[3-6] and every 1.0 mmol/L reduction in LDLc is associated with a corresponding 20-25% reduction in CVD mortality and non-fatal myocardial infarction[7].

Based on the literature, diabetes guidelines have incorporated targets for LDLc as well as recommendations for pharmacological and lifestyle treatment to reach these targets[8,9]. However, several studies show that in clinical practice, a large proportion (44-67%) of patients with type 2 diabetes mellitus does not achieve the recommended treatment targets[10-12], but an integral understanding of where opportunities may lie to improve management are lacking[13]. Whereas some studies on this subject focus on pharmacological management and general lifestyle habits (i.e. body mass index (BMI), smoking, alcohol), and others on in-depth nutritional habits[14-16], an integral approach is warranted, because both pharmacological treatment and dietary composition contribute to clinical outcomes, and are part of clinical management. Previously we have shown that for blood pressure management there are numerous opportunities in lifestyle management[17].

In this study, we aim to determine the prevalence of LDLc target achievement in a real-life population of type 2 diabetes mellitus patients in secondary care, and investigate whether in those not on target, there is room for intensifying pharmacological and lifestyle management according to current treatment guidelines.

## Materials and methods

We performed a cross-sectional analysis in baseline data from the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1). The study population and study procedures of DIALECT-1 have been described previously[17]. DIALECT is an observational cohort study in patients with type 2 diabetes mellitus, that was designed to study associations between lifestyle habits and clinical outcomes. The study has been approved by local institutional review boards (METC-Twente, NL57219.044.16; METC-Groningen, 1009.68020), is registered in the Netherlands Trial Register (NTR trial code 5855) and is performed according to the guidelines of good clinical practice and the declaration of Helsinki as revised in 2008. All participants signed an informed consent form prior to participation. The reporting of the study conforms to the STROBE statement[18].

*Setting*

Between September 2009 and January 2016, a total of 450 high-risk type 2 diabetes patients were included in DIALECT-1, the flowchart of inclusion was previously described[17]. DIALECT-1 was performed in the outpatient clinic internal medicine of the Ziekenhuisgroep Twente (ZGT) Hospital, Almelo and Hengelo, the Netherlands. The ZGT hospital is a secondary care center for diabetes treatment. In the Netherlands, referral criteria to secondary health care are: inability to achieve adequate glycaemic control with oral antidiabetic drugs or a standard insulin regimen, overt nephropathy (macroalbuminuria and/or estimated glomerular filtration rate (eGFR) below 60 ml/min•1.73m$^2$), or multiple cardiovascular complications.

*Participants*

All patients, aged 18+ years, visiting the internal medicine outpatient clinic for type 2 diabetes mellitus treatment were eligible for the study. Exclusion criteria were inability to understand the informed consent procedure, insufficient command of the Dutch language, or renal replacement therapy. Eligible patients were selected from the electronic patient file and contacted by phone.

*Variables*

At the clinic, sociodemographic characteristics, medical history, lifestyle behaviors, and current medications were recorded and anthropometric dimensions were measured using standard procedures. In clinical practice, upon initiation of statin therapy, nutraceutical use is extensively discussed as an alternative option to reduce LDLc, and thereafter is recorded in the electronic patient file. As we found no to very few mentions (<1%) of nutraceutical use in the patients' files, nutraceutical use was not included in this study. Medical history was additionally reviewed in the hospital electronic patient files on three

different occasions, by three different physician researchers. Macrovascular disease was defined presence of either coronary heart disease, cerebrovascular disease or peripheral artery disease. Coronary heart disease (CHD) was defined as presence of one of the following in medical history: physician diagnosed unstable angina pectoris, myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft. Cerebrovascular disease was defined as a history of transient ischemic attack or cerebrovascular accident. Peripheral artery disease was defined as presence of one of the following in medical history: proven artery disease by angiogram or magnetic resonance angiogram, percutaneous transluminal angioplasty or peripheral artery bypass graft.

Blood pressure was measured in a supine position by an automated device (Dinamap®; GE Medical systems, Milwaukee, WI) for 15 minutes with a one-minute interval. The mean systolic and diastolic pressure of the final three measurements was used for further analysis.

Physical activity was assessed using the Short QUestionnaire to ASses Health enhancing physical activity (SQUASH) questionnaire, which was previously validated and is commonly used in the Netherlands for population research[19]. Diet was assessed using a semi-quantitative Food-Frequency Questionnaire (FFQ) inquiring about intake of 177 items during the last month, taking seasonal variations into account[20]. The FFQ was developed and validated at the Wageningen University and has been updated several times[20,21]. For each item, the frequency was recorded in times per day, week, or month. The number of servings was expressed in natural units (e.g., slice of bread or apple) or household measures (e.g., cup or spoon). Both questionnaires were self-administered and filled out at home. The filled in questionnaires were checked for completeness by a trained researcher, and inconsistent answers were verified with the patients. Dietary data were converted into daily nutrient intake using the Dutch Food Composition Table of 2013[22]. Patients with a very low (<500 kcal/day) or very high (>6000 kcal/day) were excluded from the analyses, which was based on habitual caloric intake in the Netherlands[23].

Blood was drawn from venipuncture in a non-fasting state, for measurement of cholesterol and other variables relevant for diabetes. Total, HDL cholesterol and triglycerides were determined with the enzymatic colorimetric method using routine laboratory procedures with a Clinical Chemistry Analyzer & Immunochemistry Analyzer (COBAS 8000; Roche Diagnostics GmbH, Mannheim, Germany). LDL cholesterol was calculated using the Friedewald formula (only if triglycerides <4.5 mmol/l). 24-hour urine collections were performed as prescribed previously[17].

*Targets and definitions*
The treatment target for LDLc was set as ≤2.5 mmol/l, according to the Dutch guidelines for cardiovascular risk management in type 2 diabetes mellitus used by internists[9]. As the European guideline for cardiovascular disease prevention defines the target LDLc at <1.8 mmol/l[8] for patients with a very high risk (97% of our population), and this target is used by Dutch cardiologists, we also studied how well this target was reached.

According to the general Dutch guidelines for cardiovascular risk management used by internists, statin therapy is indicated when LDLc is >2.5 mmol/l and the 10-year risk of cardiovascular disease is ≥20%, or the risk is 10-20% and there is an additional risk factor (i.e. family member with CVD, physical inactivity, BMI≥30 or reduced renal function)[9]. The 10-year risk is determined using age, smoking status, systolic blood pressure, and the total cholesterol/HDLc ratio. In type 2 diabetes mellitus patients, 15 years should be added to the patients' age before calculating the risk. The first treatment step is simvastatin 40 mg/day, followed by atorvastatin 20-80 mg/day, or rosuvastatin 10-40 mg/day, according to maximal tolerated doses and LDLc response.

In our study, medium intensity statin treatment was defined as: simvastatin 20-40 mg/day, atorvastatin 10-20 mg/day, rosuvastatin 5 mg/day and pravastatin 40-80 mg/day[24]. Lower and higher prescribes dosages of the above-mentioned statins were defined as low intensity and high intensity statin treatment respectively.

General lifestyle recommendations were BMI ≤25 kg/m$^2$ and smoking cessation[9]. The recommendation for physical activity was at least 5 days per week 30 minutes of moderate-vigorous exercise (such as cycling, brisk walking, gardening)[9]. Dietary recommendations were derived from the Dutch dietary guidelines 2015 published by the Health Council of the Netherlands[25], which are also adopted by the Dutch Diabetes Federation and used in clinical practice by dieticians treating diabetes patients[26]. In short the recommended intakes were: vegetables ≥200 g/day; fruits ≥200 g/day; wholegrain products ≥90 g/day; legumes ≥1 portion/week; unsalted nuts ≥15 g/day; low-fat dairy 2-3 portions/day (including milk or yoghurt); fish ≥1 portion/week; flack or green tea ≥3 cups/day; use soft margarines, liquid cooking fats and vegetable oils instead of butter or hard margarines and cooking fats; replace unfiltered coffee by filtered coffee; red meat ≤45 g/day; no processed meat; no consumption of sweetened beverages and fruit juices; alcohol ≤1 unit/day; sodium ≤2.3 g/day. As data on whether consumed grains were wholegrain or refined, and data on whether consumed coffee was filtered or unfiltered were not available from the FFQ used in our study, these components were not analyzed here. Recommended daily legume and fish intake was calculated by dividing one portion size (60g) by 7 and rounding up to 10 g/day. Because the FFQ did not distinguish between salted and unsalted

nuts, and type of tea, total nut intake and total tea intake respectively were used in our calculations. Data on dietary sodium intake was derived from the 24h urinary sodium excretion[17].

*Statistics*

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS; IBM, Chicago, IL, USA), version 22.0. Normality of data was assessed by visually inspecting the frequency histograms. Normally distributed data were presented as mean ± standard deviation. Skewed variables were expressed as median [interquartile range]. Dichotomous variables were presented in number and percentage. Cases with missing data were excluded from the respective analyses. To describe characteristics of patients who had achieved different LDLc values, the population was divided in three groups according to LDLc <1.8 mmol/l, 1.8-2.5 mmol/l and >2.5 mmol/l. Differences between the groups were tested using one-way ANOVA (normally distributed), Kruskal Wallis (skewed) or Chi Square (categorical).

# Results

*Baseline characteristics*

For the current study plasma LDLc concentrations were available in 428 patients of the total 450 patients included in DIALECT-1. Baseline characteristics are shown in Table 1. Mean age of patients was 63 ± 9 years, and 58% of patients were men. The median diabetes duration was 11 [7-18] years, mean HbA1c was 57 ± 12 mmol/mol (7.4 ± 3.2%). Most patients were overweight or obese, mean BMI was 32.0 ± 6.3 kg/m$^2$ and 6% of patients had a BMI <25kg/m$^2$. The majority of patients had one or more complications: 65% of patients had microvascular disease, with nephropathy being the most frequent (42% of all patients), and 35% had macrovascular disease.

Mean LDLc in the whole population was 2.0 ± 0.8 mmol/l. In total 334 patients (78%) achieved the target LDLc ≤2.5 mmol/l, among which 184 patients (43% of the total population) achieved an LDLc <1.8 mmol/l (Table 1). Patients with LDLc ≤2.5 mmol/l had a longer diabetes duration (P=0.006) and more often used insulin (P=0.02). Furthermore, patients who were on target LDLc ≤2.5 mmol/l more often had retinopathy (P=0.001).

*Pharmacological lipid lowering therapy and LDLc target achievement*

Of all patients, 76% were on current statin therapy (Table 1). The most prevalent reasons for non-treatment were "not indicated according to guideline" and "previously reported side-effects/patient preference" (both 8% of the total population). In 7% of patients the reason for not using a statin was not documented in the patient file, and there was no documentation of previous statin use. Of the 88 patients not on statin therapy, and with an indication for lipid lowering therapy (LLT), 15 patients used ezetimibe and 6 patients used fibrates.

Of patients on target LDLc (≤2.5 mmol/l), 83% used statins, while 41% of patients with an LDLc >2.5 mmol//l used statins. High intensity statin treatment was the most prevalent in patients with a LDLc <1.8 mmol/l (23%), versus 13% in those with LDLc 1.8-2.5 mmol/l and 8% in those with LDLc >2.5 mmol/l (figure 1). Of patients who did not achieve the target LDLc of ≤2.5 mmol/l, 46% did not use any LLT, while 5% used low intensity and 28% used moderate intensity statin treatment. Of the 54 non-statin users in the LDLc >2.5 mmol/l group, 24 patients experienced side-effects or had a personal preference to avoid statins, and in 23 patients the reason for not using statins was not documented.

**Table 1**. Patient characteristics categorized by LDLc target groups

| | Total population | LDLc <1.8 | LDLc 1.8-2.5 | LDLc >2.5 | |
|---|---|---|---|---|---|
| *Number of patients (% of total population)* | *428* | *184 (43)* | *150 (35)* | *94 (22)* | *P-value* |
| Age, years | 63 ± 9 | 64 ± 9 | 62 ± 9 | 62 ± 10 | 0.10 |
| Male, n (%) | 248 (58) | 117 (64) | 79 (55) | 52 (52) | 0.11 |
| Duration of diabetes, years | 11 [7-18] | 13 [8-20] | 11 [6-18] | 10 [5-14] | 0.006 |
| Serum HbA1c, mmol/mol | 57 ± 12 | 58 ± 11 | 56 ± 12 | 58 ± 13 | 0.12 |
| Serum HbA1c, % | 7.4 ± 3.2 | 7.5 ± 3.2 | 7.3 ± 3.2 | 7.5 ± 3.3 | 0.12 |
| Insulin use, n (%) | 271 (63) | 129 (70) | 88 (62) | 54 (54) | 0.02 |
| Systolic blood pressure, mmHg | 136 ± 16 | 136 ± 17 | 137 ± 16 | 136 ± 17 | 0.70 |
| Diastolic blood pressure, mmHg | 74 ± 10 | 73 ± 10 | 75 ± 9 | 75 ± 10 | 0.17 |
| Heart frequency, beats/min | 74 ± 13 | 73 ± 13 | 75 ± 12 | 74 ± 12 | 0.52 |
| Antihypertensive treatment, n (%) | 347 (81) | 159 (86) | 113 (79) | 75 (74) | 0.32 |
| Total number of drugs | 7 ± 3 | 7 ± 3 | 7 ± 3 | 6 ± 3 | 0.02 |
| Microvascular disease, n (%) | 280 (65) | 135 (74) | 85 (60) | 60 (61) | 0.008 |
| Diabetic nephropathy, n (%) | 178 (42) | 90 (49) | 50 (35) | 38 (38) | 0.02 |
| Retinopathy, n (%) | 103 (24) | 59 (32) | 32 (23) | 12 (12) | 0.001 |
| Neuropathy, n (%) | 155 (36) | 70 (38) | 42 (29) | 43 (43) | 0.03 |
| Macrovascular disease, n (%) | 149 (35) | 78 (42) | 38 (27) | 33 (33) | 0.007 |
| Coronary heart disease, n (%) | 93 (22) | 49 (27) | 22 (15) | 22 (22) | 0.04 |
| Cerebrovascular disease, n (%) | 46 (22) | 25 (14) | 13 (9) | 8 (8) | 0.26 |
| Peripheral artery disease, n (%) | 42 (10) | 26 (14) | 4 (3) | 12 (12) | 0.003 |
| Total cholesterol, mmol/l | 4.0 ± 0.9 | 3.3 ± 0.5 | 4.1 ± 0.4 | 5.0 ± 0.7 | <0.001 |
| LDL-cholesterol, mmol/l | 2.0 ± 0.8 | 1.4 ± 0.3 | 2.1 ± 0.2 | 3.1 ± 0.5 | <0.001 |
| HDL-cholesterol, mmol/l | 1.1 ± 0.3 | 1.1 ± 0.3 | 1.2 ± 0.4 | 1.1 ± 0.3 | 0.04 |
| Total cholesterol/HDL ratio, mmol/l | 3.8 ± 1.4 | 3.2 ± 1.0 | 3.7 ± 1.1 | 4.9 ± 1.7 | <0.001 |
| Triglycerides, mmol/l | 1.8 ± 0.9 | 1.9 ± 1.0 | 1.8 ± 0.8 | 1.9 ± 0.8 | 0.04 |
| C-reactive protein, mg/l | 2 [1-5] | 2 [1-5] | 2 [1-5] | 3 [1-6] | 0.13 |
| *Pharmacological lipid lowering therapy* | | | | | |
| Statin, n (%) | 324 (76) | 169 (92) | 114 (76) | 41 (44) | <0.001 |
| Ezetimibe, n (%) | 34 (8) | 9 (5) | 10 (7) | 15 (16) | 0.004 |
| Statin + ezetimibe, n (%) | 22 (5) | 8 (4) | 6 (4) | 8 (9) | 0.16 |
| Fibrate, n (%) | 10 (2) | 0 (0) | 4 (3) | 6 (6) | 0.003 |
| Statin + fibrate, n (%) | 4 (1) | 0 (0) | 2 (1) | 2 (2) | 0.17 |
| Other lipid lowering therapy, n (%) | 1 (0) | 0 (0) | 1 (1) | 0 (0) | 0.40 |
| No lipid lowering therapy, n (%) | 87 (20) | 14 (8) | 31 (21) | 42 (45) | <0.001 |
| Glucagon-like peptide-1 analogues, n (%) | 43 (10) | 14 (8) | 20 (13) | 9 (10) | 0.22 |

**Table 1**. Continued

| | Total population | LDLc <1.8 | LDLc 1.8-2.5 | LDLc >2.5 | |
|---|---|---|---|---|---|
| *Number of patients (% of total population)* | *428* | *184 (43)* | *150 (35)* | *94 (22)* | *P-value* |
| *Lifestyle and nutritional factors* | | | | | |
| BMI, kg/m² | 32.0 ± 6.3 | 33.0 ± 6.3 | 33.1 ± 6.0 | 32.4 ± 6.8 | 0.16 |
| BMI ≤25 kg/m², n (%) | 24 (6) | 7 (4) | 8 (5) | 9 (10) | 0.14 |
| Waist circumference, cm | 112 ± 14 | 113 ± 14 | 112 ± 14 | 108 ± 14 | 0.02 |
| Current smoker, n (%) | 72 (17) | 20 (20) | 28 (15) | 24 (17) | 0.57 |
| Adherence guideline physical activity, n (%) | 244 (59) | 97 (56) | 92 (63) | 55 (60) | 0.46 |
| Total caloric intake, kcal/day | 1922 ± 636 | 1922 ± 645 | 1958 ± 628 | 1865 ± 632 | 0.56 |
| Vegetables, g/day | 98 [57-136] | 95 [49-124] | 99 [56-146] | 101 [72-136] | 0.26 |
| Fruits, g/day | 123 [66-227] | 120 [66-218] | 130 [73-226] | 116 [66-232] | 0.68 |
| Legumes, g/day | 12 [0-27] | 17 [4-28] | 11 [0-24] | 11 [0-30] | 0.34 |
| Nuts, g/day | 3 [0-9] | 3 [0-6] | 4 [0-11] | 4 [0-10] | 0.05 |
| Dairy, g/day | 213 [104-357] | 231 [118-343] | 207 [87-394] | 167 [89-289] | 0.10 |
| Fish, g/day | 10 [3-20] | 10 [3-21] | 11 [4-19] | 10 [2-17] | 0.72 |
| Tea, g/day | 71 [0-250] | 71 [0-250] | 71 [0-250] | 125 [9-250] | 0.12 |
| Butter, hard margarines and cooking fats, g/day | 0 [0-7] | 0 [0-7] | 0 [0-7] | 0 [0-5] | 0.73 |
| Red meat, g/day | 91 [66-117] | 92 [69-117] | 93 [69-125] | 85 [58-110] | 0.13 |
| Processed meat, g/day | 48 [30-72] | 49 [30-73] | 50 [33-70] | 44 [26-65] | 0.38 |
| Sweetened beverages and fruit juices, g/day | 27 [0-129] | 21 [0-127] | 27 [0-129] | 29 [0-105] | 0.88 |
| Alcohol, units/month | 5 [0-31] | 3 [0-37] | 8 [0-30] | 4 [0-25] | 0.50 |
| Sodium, g/day | 4.3 ± 1.8 | 4.2 ± 1.7 | 4.4 ± 2.0 | 4.1 ± 1.8 | 0.41 |

LDLc, low density lipoprotein cholesterol; BMI, body mass index. Differences between the groups are determined using one-way ANOVA (normal distribution), Kruskal Wallis (skewed distribution) or chi square (categorical variables)

*Lifestyle and LDLc target achievement*

Adherence to general lifestyle guidelines was low in the overall population, and was not different between the LDLc target groups (Table 1). In the overall population, 6% had a BMI ≤25kg/m² and 59% had physical activity as recommended. Smoking guidelines were followed relatively well, as 83% were either non-smokers or former smokers.

The mean total kilocaloric (kcal) intake per day was 1922 ± 636 kcal/day, and was not different between the LDLc target groups (Table 1). There were no differences in absolute intake of dietary products among the LDLc groups. Median vegetable intake was 98 [57-136] g/day and median fruit intake was 123 [66-227] g/day.

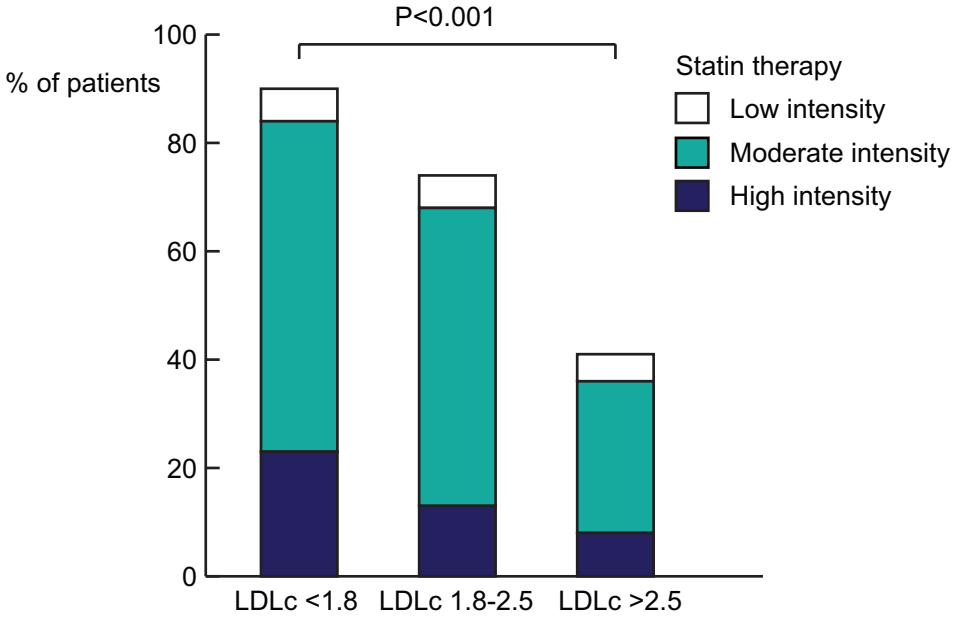


**Figure 1**. Intensity of statin treatment in LDLc groups. There was a significant difference in intensity of statin use between the LDLc groups. Medium intensity statin treatment was defined as: simvastatin 20-40 mg/day, atorvastatin 10-20 mg/day, rosuvastatin 5 mg/day and pravastatin 40-80 mg/day. Lower and higher prescribes statin dosages were defined as low intensity and high intensity respectively.

Adherence to dietary guidelines was low in the whole population and there were no differences between the LDLc target groups (figure 2). Only 7% of the population consumed ≥200g vegetables per day, while 28% consumed ≥200g fruit per day. Furthermore, 59% of the population consumed legumes once weekly, 14% ate ≥15g nuts per day and 19% consumed 2-3 portions of low-fat dairy per day. Fish intake was as recommended in 36% of the population, and 8% drank tea as recommended. Adherence to fats and oils intake was reasonably well with 66%. In regard to meat consumption, 12% did not eat more red meat than recommended and 2% ate no processed meats. Alcohol intake was one unit per day or less in 71%, and 34% drank no sweetened beverages. Sodium intake was below 2.3 g/day in 12% of the population.

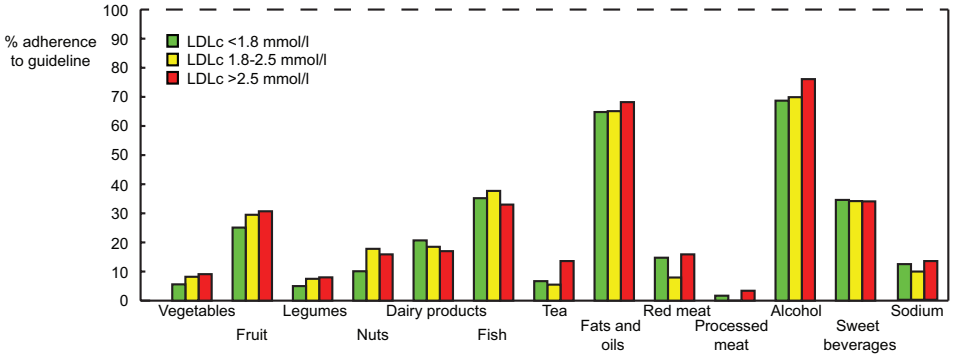


**Figure 2.** Adherence to dietary guidelines on nutritional intake. There were no differences in adherence rates between the LDLc target groups.

## Discussion

In this real-life study in type 2 diabetes mellitus with high cardiovascular risk, the target LDLc of ≤2.5 mmol/l is reached in three quarters of the patients. Statin use was markedly more frequent and statin dosage was higher in patients who had achieved the LDLc target. Therefore, pharmacological treatment with statins, used in approximately three quarters of patients, is the most important part of LDLc lowering treatment in routine clinical practice. In contrast, adherence to lifestyle guidelines was poor in the whole study population, especially on BMI and intake of vegetables, legumes, nuts, red and processed meat, tea and sodium.

Overall, the data illustrate that statin therapy is well incorporated in routine diabetes care, and is an effective tool to reach target LDLc in the real world setting of type 2 diabetes mellitus treatment. The percentage of adequately controlled LDLc reported here is somewhat higher than found in other studies. A previous study demonstrated LDLc target achievement in 56% of type 2 diabetes mellitus patients treated in the primary health care setting in the Netherlands[11]. In the large European EUROASPIRE study, LDLc targets attainment was reported in 33% of patients[12]. De Cosmo at al. reported adequately controlled LDLc in 51% of patients with diabetes and chronic kidney disease[10]. The high percentage of patients on target for LDLc we found, illustrates that lipid lowering therapy is well-incorporated in routine secondary clinical care of high-risk type 2 diabetes mellitus patients. The target LDLc was more often reached by patients with more serious disease, as indicated by a longer duration of type 2 diabetes mellitus, more frequent insulin use, and higher prevalence of microvascular complications. This suggests that a higher urgency for aggressive LDLc lowering treatment is experienced in patients with a higher grade of co-morbidity.

In the patients who were not on statin treatment, one third had no strict indication for lipid lowering therapy, one third had previously experienced side-effects, and in roughly one third, the reason for not using a statin was not documented. Possibly, the latter subgroup consists of individuals with a preference of not using a statin, either based on general perceptions or because of side-effects in the past (this was not documented in the patient files). The chance that the option of prescribing a statin has been overlooked is negligible, taking into account the disease duration, frequent contact with sequential physicians and diabetes nurses and a system in which also pharmacists verify adherence to guidelines.

Adherence to dietary recommendations was low in our population of type 2 diabetes mellitus patients. It should be noted that the majority of findings on dietary intake reported here were not different from dietary intake in the general Dutch population as reported by the National Institute for Public Health [27-29]. The general population had a slightly lower intake of fruits (113 vs 123 g/day in DIALECT), legumes (5 vs 12 g/day in DIALECT), red meat (79 vs 91 g/day in DIALECT) and low-fat dairy (180 vs 213 g/day in DIALECT). Intake of processed meat (50 vs 49 g/day in DIALECT) and fish (18 vs 10 g/day in DIALECT) was comparable. Intake of vegetable was higher in the general population, 139 g/day vs 98 g/day in DIALECT. Interestingly, intake of sweetened beverages was substantially higher in the general population (336 vs 27 g/day in DIALECT). The difference in intake of sweetened beverages possibly reflect the effect of dietary counselling in diabetes patients. In conclusion, it is important to recognize that non-adherence to dietary guidelines is not a problem specific for type 2 diabetes mellitus patients, but is a population-wide phenomenon.

It should be noted that the LDLc target in Dutch secondary care is defined as ≤2.5 mmol/l, both for high-risk and very high-risk patients[9]. This is different from the European guidelines, where the LDLc target is <1.8 mmol/l for very high-risk patients (97% of our population)[8]. When using European guidelines, target achievement was somewhat lower (43%). However, when using these guidelines, results of analyses on where treatment opportunities lie to improve target achievement were similar: in patients not on target, high intensity statin use was infrequent (<15%) and adherence to lifestyle guideline adherence was low in all LDLc groups.

On a side note, we found a relatively high grade of adherence to the recommendation for physical activity (59%), in the general Dutch population this number was 54% in 2015[30]. In a systematic review, correlations between subjectively and objectively measured physical activity were weak to moderate[31]. This poses the question if the compliance to the recommendation we report here is an accurate reflection of the actual physical activity of our population.

This study has several strengths. The integration of pharmacological and lifestyle parameters collected in a real-life setting provides the best possible tool to define opportunities to improve treatment strategies in type 2 diabetes mellitus. In addition, our study population represents a real-life clinical setting, where inclusion bias was minimal due to the broad inclusion criteria. A potential limitation of the study is that venipuncture was not performed in the fasting state[8], leading to a possible overestimation of serum triglycerides and therefore underestimation of LDLc levels. However, serum triglycerides were not higher than expected and therefore we estimate that this effect was minimal. Also, the

use of the food frequency questionnaires might lead to underestimation of intake of unhealthy products in dietary intake. Nevertheless, there are currently no better methods for registration of dietary habits in a study with this size. Lastly, the cross-sectional design only allows to study associations, and not causality. Future prospective studies are necessary to evaluate the effects of increasing pharmacological treatment and increasing lifestyle guideline adherence on LDLc target achievement.



What should be the implications of this study? We found that pharmacological treatment with statins was substantially higher in patients who had reached the LDLc target. Therefore, in patients who are not on target LDLc, the first step could be to explore the opportunities to either start or intensify statin treatment. As opposed to blood pressure management in this cohort, where therapy resistance is an issue in roughly 40% of the patients not on target blood pressure[17], our data suggest that resistance to statin treatment is less common. In those with an LDLc not on target, only 8% were on high intensity statin treatment. High intensity statin treatment can reduce LDLc by 40-60%, versus a 20-30% reduction on low intensity treatment[32]. Therefore, intensifying statin treatment, either by increasing the dose, or by switching to a more potent compound, could be an option in a large subset of patients not on target. Also, adherence to therapy should be addressed. Relatively low adherence to statin therapy has been previously reported[33-35], especially after negative reports on statins by the mainstream media[36,37], rendering it worthwhile to investigate whether prescribed statins are actually ingested by the patients. Unfortunately, such data were not available here. It should be noted that statin treatment efficacy can vary considerably per individual, in literature a range of 5-70% LDLc reduction in different individuals is reported[38,39]. Statin treatment resistance, i.e. not achieving LDLc target despite high intensity statin treatment, has been associated with black racial ancestry, polymorphisms in genes affecting statin pharmacodynamics and pharmacokinetics, smoking, inflammation and HIV-infection[40]. Certain drugs may also reduce statin effectiveness by reducing bioavailability (i.e. bile acid sequestrants) or increasing statin metabolism (i.e. rifampicin). Nutraceuticals, which sometimes are used in the general population, might also influence LDLc target achievement, however few studies have been performed on the interaction between statin use and nutraceuticals, and therefore the effect of nutraceuticals statin efficacy remains unclear[41]. In addition, clinical conditions that increase cholesterol levels, such as hypothyroidism, should also be reviewed. However, in a real-life setting, LDLc target non-achievement despite statin treatment, can most often be attributed to treatment non-adherence, rather than treatment resistance[42,43].

For patients who fail to reach their LDLc target, there are alternative pharmacological options to reduce LDLc. First, in patients who do not achieve the target LDLc despite maximum-tolerated dose statin use, ezetimibe therapy should be considered[8,44]. We

found that only a quarter of not on target patients used ezetimibe, with or without concurrent statin use, and therefore ezetimibe therapy could be increased. In the case of mixed dyslipidemia (i.e. high LDLc and high triglycerides), fibrate therapy, which was used by 8% of patients not on LDLc target in this population, could also be considered[45]. When both maximum-tolerated statin use and ezetimibe are insufficient, the relatively new drug class of PCSK9 inhibitors (not used in this population) has the potential to reduce LDLc by 32-71%, depending on the used dosage[46,47]. In addition, glucagon-like peptide-1 (GLP-1) analogues, which improve glycaemic regulation, body weight and blood pressure in type 2 diabetes mellitus, have also been shown to reduce LDLc and triglycerides, and increase HDLc[48-50]. Moreover, GLP-1 analogues have a favorable effect on total cardiovascular risk: in the recent LEADER trial, cardiovascular death was 22% lower in type 2 diabetes mellitus patients treated with liraglutide, compared to placebo-treated patients[51]. Therefore, the use of GLP-1 analogues in clinical practice should not be overlooked, especially in those with poor glycaemic regulation in combination with obesity, hypertension or dyslipidemia[52].

Alternatively, especially in the patients that have a personal preference not to use statins or are intolerant to statins, lifestyle intervention could be a worthwhile option to improve LDLc and improve general cardiovascular risk management. With respect to diet, one could consider to focus on increasing the intake of vegetables and legumes, and reducing the intake of red and processed meat. It has previously been shown in patients not on statin therapy, dietary changes can reduce LDLc by 13-30%[53-55]. In a post-hoc analyses of the Alpha Omega Trial, in which the effect of omega 3 fatty acid supplementation on cardiovascular outcomes was studied in post-myocardial infarction patients, a beneficial effect was shown in those not on current statin treatment[56]. It should be noted that little data is available on the extent of LDLc reduction through dietary changes in patients already on statin treatment. Stakeholders of nutritional research in the Netherlands have hypothesized that the high efficacy of pharmacological therapy is one of the reasons that lifestyle intervention is less emphasized in clinical care[57]. This is supported by our finding that statins, and not lifestyle, are the main determinants of LDLc control. Nevertheless, adopting a healthy lifestyle has pleiotropic effects not only on cholesterol, but also on other cardiovascular risk factors such as obesity, blood pressure and insulin resistance, for which treatment resistance is a growing concern [58-63]. For example, a recent meta-analysis of 39 studies has shown that physical exercise can reduce LDLc and improve insulin sensitivity[64], especially in obese subjects. However, adherence to dietary guidelines on these compounds is low in the overall population, illustrating that community intervention might be more appropriate, then just targeting type 2 diabetes mellitus patients. In the meantime, focus should be placed to promote a healthy diet and lifestyle in patients with type 2 diabetes mellitus.

## Conclusion

In this population of high-risk type 2 diabetes mellitus patients in a real-world secondary health care, the LDLc target is achieved by the majority of the population. High intensity statin treatment was infrequent in patient who did not achieve the LDLc target, therefore, a good opportunity to improve LDLc target achievement could be to put as many as possible patients on appropriate dose of statins. Nevertheless, adherence to lifestyle and dietary recommendation is low, especially on BMI and intake of vegetables, legumes, nuts, red and processed meat, tea and sodium. Therefore, the focus on lifestyle intervention remains of great importance, because of multiple beneficial effects on obesity, blood pressure and insulin resistance.



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# CHAPTER 4

## Glycaemic Control in the DIAbetes and LifEstyle Cohort Twente - Integrated Assessment of Lifestyle and Pharmacological Management on Ideal HbA1c Target Achievement

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# Abstract

**Objective** In cardiovascular risk management, the majority of patients with type 2 diabetes does not reach target glycated hemoglobin (HbA1c <7%). We determined the prevalence of HbA1c target achievement in a real-life population of patients with type 2 diabetes in secondary care. To identify opportunities for improving HbA1c target achievement, we assessed adequacy of lifestyle and pharmacological treatment.

**Research design and methods** We performed cross-sectional analyses in baseline data from the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1, *n*=450, age 63 ± 9 years, 58% men, diabetes duration of [7-18] years). Patients were divided according to 1) HbA1c <7% (<53 mmol/mol) and HbA1c ≥7% (≥53 mmol/mol), and 2) non-insulin treatment and tertiles of daily insulin use.

**Results** Mean HbA1c was 7.4% ± 3.2% (57 ± 12 mmol/mol), and 161 (36%) patients achieved the target HbA1c of <7% (<53 mmol/mol). Patients with HbA1c ≥7% had a longer diabetes duration (13 [8-20] vs 9 [4-14] years, P<0.001) and more frequently were insulin-users (76% vs 41%, P<0.001). Lifestyle guidelines adherence was low, and was not different between the HbA1c groups. Patients in the highest tertile of insulin use had a higher body mass index than those in the lowest tertile (35.8 ± 5.5 vs 29.8 ± 5.5, kg/m$^2$, P<0.001).

**Conclusions** Target HbA1c achievement is low in this real-life population of patients with type 2 diabetes. Pharmacological treatment resistance was high, and was paralleled by a high body mass index. Increasing insulin sensitivity through lifestyle intervention is the best opportunity to improve HbA1c target achievement.

# Introduction

Type 2 diabetes mellitus is a highly prevalent disease, with over 425 million patients worldwide, and is associated with substantial morbidity and mortality[1]. Prevention of microvascular complications and cardiovascular risk management (CVRM) are the cornerstones of treatment in type 2 diabetes, as in the course of the disease, 29% of patients develops vision threatening retinopathy, 40% of patients develops nephropathy, and even 60% develops one or more cardiovascular complications[2-4].

It has been well established that tight glycemic control reduces the risk of microvascular complications, and to a lesser extent also of cardiovascular disease, where each 1% (11 mmol/mol) of mean HbA1c reduction has been associated with a 21% risk reduction for any diabetes-related complications[5-7]. As too strict glycemic regulation (target HbA1c <6%; <42 mmol/mol) has been associated with increased mortality compared to standard treatment (target HbA1c <7.5%; <58 mmol/mol)[8], a target HbA1c of <7% (<53 mmol/mol) has been formulated as optimal in diabetes guidelines, and a more liberal target may be accepted in specific groups[9-11]. Strategies to achieve this target exist of lifestyle intervention and pharmacological treatment.

Unfortunately, however, a recent meta-analysis demonstrated that HbA1c target achievement (with most studies used the target <7%; <53 mmol/mol) is low, with a pooled average of 43% worldwide[12], both in primary and in secondary care settings. This low target achievement has remained unchanged in the past decade[12,13]. The reason for ongoing low target achievement, despite the expanding arsenal of glucose lowering interventions, remains to be elucidated, but physician inattentiveness, a shift in type 2 diabetes patients demographic towards younger patients, poor access to health care and low adherence to pharmacological treatment have been implicated[13-15]. Although both lifestyle and pharmacological management contribute to glycemic control, there are few studies which address both aspects of treatment in relation to HbA1c target achievement.

CVRM consist of multiple targets: blood pressure, lipids and HbA1c. Previously, we demonstrated that an integrated approach is warranted when studying opportunities for improving target achievement, as opportunities to improve clinical target achievement differ per clinical target[16,17]. In patients not on target blood pressure, there was pharmacological undertreatment in 62%, while for LDL cholesterol, this number was 92% of patients. For both target, lifestyle treatment was suboptimal in 97% of patients not on target. However, for HbA1c target achievement, the opportunities in lifestyle and pharmacological management are unknown.

Therefore, in this study we aim to 1) investigate the prevalence of ideal HbA1c target achievement in a real-life population of type 2 diabetes patients in secondary health care, and 2) identify opportunities for improving ideal HbA1c target achievement, using an integrated assessment of lifestyle factors and pharmacological treatment. It should be noted that we study a type 2 diabetes population in secondary health care in the Netherlands, where inability to achieve adequate glycemic control with oral antidiabetic drugs or a standard insulin regimen, is a major criterium for referral from primary to secondary care.

## Materials and Methods

This was a cross-sectional study in baseline data from the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1). The study population and study procedures of DIALECT-1 have been described previously[16]. The study has been approved by local institutional review boards (METC-Twente, NL57219.044.16; METC-Groningen, 1009.68020), is registered in the Netherlands Trial Register (NTR trial code 5855) and is performed according to the guidelines of good clinical practice and the declaration of Helsinki. All participants signed an informed consent form prior to participation.

*Setting*

Between September 2009 and January 2016, in total 450 type 2 diabetes patients were included in DIALECT-1, the flowchart of inclusion was previously described[16]. DIALECT-1 was performed in the outpatient clinic internal medicine of the Ziekenhuis Groep Twente (ZGT hospital), located in the cities Almelo and Hengelo, the Netherlands. The ZGT hospital is a secondary care center for diabetes treatment. In the Netherlands, referral criteria from primary to secondary health care are: inability to achieve adequate glycemic control with oral antidiabetic drugs or a standard insulin regimen, macroalbuminuria and/or estimated glomerular filtration rate (eGFR) decline below 60 ml/min, or multiple cardiovascular complications.

*Participants*

All patients, aged 18+ years, visiting the internal medicine outpatient clinic for type 2 diabetes mellitus treatment were eligible for the study. Exclusion criteria were inability to understand the informed consent procedure, insufficient command of the Dutch language, or dialysis dependency. Eligible patients were selected from the electronic patient file and contacted by phone.

*Variables*

At the clinic, sociodemographic characteristics, medical history (i.e. major adverse cardiovascular events, peripheral artery disease and microvascular complications), lifestyle behaviors, and current medications were recorded and anthropometric dimensions were measured using standard procedures. Medical history and current drug use, with special attention to glucose lowering drugs, were additionally reviewed in the hospital electronic patient files on three different occasions, by three different physician researchers.

Physical activity was assessed using the Short QUestionnaire to ASses Health enhancing physical activity (SQUASH) questionnaire, which was previously validated and is commonly used in the Netherlands for population research[18]. Diet was assessed using a

semi-quantitative validated food-frequency questionnaire (FFQ) inquiring about intake of 177 items during the last month, taking seasonal variations into account[19]. The FFQ was developed and validated at the Wageningen University and has been updated several times[19]. For each item, the frequency was recorded in times per day, week, or month. The number of servings was expressed in natural units (e.g., slice of bread or apple) or household measures (e.g., cup or spoon). Both questionnaires were self-administered and filled out at home. The filled in questionnaires were checked for completeness by a trained researcher, and inconsistent answers were verified with the patients. Dietary data were converted into daily nutrient intake of macronutrients (i.e. carbohydrates, protein, fat) using the Dutch Food Composition Table of 2013[20]. We calculated total dietary intake of food groups included in the Dutch guidelines of a healthy diet by multiplying the frequency of consumption of each food item in that specific category by total gram intake per day, and summing across all foods in that category (Supplementary Table S1)[21]. In addition, we calculated specific carbohydrate intake from several different carbohydrate-rich food categories by multiplying the frequency of consumption of each food item in that specific category by its carbohydrate content and summing across all foods in that category (Supplementary Table S2).

Blood was drawn from venipuncture in a non-fasting state, for measurement of HbA1c and other variables relevant for diabetes. HbA1c was measured by the Roche Tina-quant 3rd generation immunoturbidimetric method, standardized according to IFCC, on a Clinical Chemistry Analyzer & Immunochemistry Analyzer (COBAS 6000, Roche Diagnostics GmbH, Mannheim, Germany). Collection of 24-hour urine was performed as prescribed previously[16].

*Targets and definitions*

Ideal HbA1c was set as <7% (<53 mmol/mol), according to the European guidelines for diabetes management in type 2 diabetes mellitus, which are adopted for use in the Netherlands[9]. Because more liberal HbA1c targets are allowed in specific groups, we also studied achievement of personalized HbA1c targets according to risk profiles[11]. Personalized HbA1c targets were adopted from a previous report by Ali *et al.*[14]: the target was <7% (<53 mmol/mol) for all patients without complication, and for those aged <45 with complications. The target was <7.5% (<58 mmol/mol) for patients aged 45-64 years with complications, and the target was <8% (<64 mmol/mol) for patients aged 65+ years with complications. For our study, we used the target of <7% (<53 mmol/mol) for patients aged <45 years without complications, as this target is used in clinical practice in the Netherlands[9]. Presence of complications was defined as established coronary artery disease, cerebrovascular disease, albuminuria or retinopathy.

Insulin regimens were categorized according to basal (only a long acting or an intermediate-acting insulin), basal bolus/plus (combination of long or intermediate-acting insulin with a short-acting insulin), or mixed (mixed insulin) regimen.

General lifestyle recommendations were body mass index (BMI) ≤25 kg/m$^2$ and smoking cessation[22]. The recommendation for physical activity was at least 5 days per week 30 minutes of moderate-vigorous exercise[22]. Dietary recommendations were derived from the "Dutch Dietary Guidelines 2015", published by the Health Council of the Netherlands[21], which are also adopted by the Dutch Diabetes Federation and used in clinical practice by dieticians treating diabetes patients[23]. In short, the recommended intakes were: vegetables ≥200 g/day; fruits ≥200 g/day; wholegrain products ≥90 g/day; legumes ≥1 portion/week; unsalted nuts ≥15 g/day; low-fat dairy 2-3 portions/day (including milk or yoghurt); fish ≥1 portion/week; flack or green tea ≥3 cups/day; use soft margarines, liquid cooking fats and vegetable oils instead of butter or hard margarines and cooking fats; replace unfiltered coffee by filtered coffee; red meat ≤45 g/day; no processed meat; no consumption of sweetened beverages and fruit juices; alcohol ≤10 gram/day; sodium ≤2.3 g/day. Wholegrains and coffee were not analyzed, ss data on whether consumed grains were wholegrain or refined, and data on whether consumed coffee was filtered or unfiltered were not available. Recommended daily legume and fish intake was calculated by dividing one portion size (60g) by 7 and rounding up to 10 g/day. Because the FFQ did not distinguish between salted and unsalted nuts, and type of tea, total nut intake and total tea intake respectively were used in our calculations. Data on dietary sodium intake was derived from the 24h urinary sodium excretion.

*Statistics*

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS; IBM, Chicago, IL, USA), version 22.0. Normality of data was assessed by visually inspecting the frequency histograms. Normally distributed data were presented as mean ± standard deviation, skewed variables as median [interquartile range], dichotomous variables as number (percentage).

Patients were divided according to HbA1c on ideal target (HbA1c-OIT; <7%; <53 mmol/mol) and HbA1c not on ideal target (HbA1c-NOIT; ≥7%; ≥53 mmol/mol). In addition, patients were divided according to personalized HbA1c target achievement. Differences between the groups were tested using student t test (normal distribution), Mann Whitney U (skewed distribution) and Chi Square (categorical).

In addition, as we found that intensity of blood glucose lowering treatment was higher in patient with HBA1c-NOIT, we studied which factors were associated with higher blood glucose lowering treatment intensity. For this end, we divided patients in four groups, where the first group was non-insulin users (i.e. patients with only non-insulin blood glucose lowering treatment), and the second to fourth groups were based on tertiles of daily used insulin units. Differences between the groups were tested using the one-way ANOVA (normal distribution), Kruskal Wallis (skewed distribution) and Chi Square (categorical).

# Results

HbA1c data were available in all of the 450 patients making up the DIALECT-1 cohort. Baseline characteristics of the population are shown in Table 1. Mean age was 63 ± 9 years, and 58% (*n*=261) of patients were men. The median type 2 diabetes duration was 11 [7-18] years. The majority of our population was overweight or obese with a mean BMI of 32.9 ± 6.2 kg/m2 and only 5% (*n*=24) of patients had a BMI <25 kg/m$^2$. The majority of the population was affected by one or more type 2 diabetes complications: 296 (67%) patients had microvascular disease and 160 (36%) macrovascular disease.

**Table 1.** Patient characteristics of DIALECT-1 by a breakup of ideal HbA1c target achievement

| *Variable* | | *Total population* | *HbA1c-OIT* | *HbA1c-NOIT* | *P-value* |
|---|---|---|---|---|---|
| | *n* | | *<7% (<53mmol/mol)* | *≥7% (≥53 mmol/mol)* | |
| *Number of patients, n (%)* | | *n=450* | *n=161 (36)* | *n=287 (64)* | |
| Age, years | *450* | 63 ± 9 | 63 ± 9 | 63 ± 9 | 0.63 |
| Men, n (%) | *450* | 261 (58) | 85 (53) | 174 (61) | 0.13 |
| Diabetes duration, years | *450* | 11 [7-18] | 9 [4-14] | 13 [8-20] | <0.001 |
| Body Mass Index, kg/m$^2$ | *448* | 32.9 ± 6.2 | 33.0 ± 6.8 | 32.8 ± 5.8 | 0.80 |
| Waist/hip ratio, cm/cm | *441* | 1.00 ± 0.09 | 0.99 ± 0.08 | 1.01 ± 0.09 | 0.09 |
| Systolic blood pressure, mmHg | *449* | 136 ± 16 | 135 ± 17 | 137 ± 16 | 0.25 |
| Diastolic blood pressure, mmHg | *448* | 74 ± 10 | 74 ± 10 | 75 ± 9 | 0.53 |
| Heart frequency, beats/min | *444* | 74 ± 13 | 74 ± 14 | 74 ± 12 | 0.98 |
| Blood pressure on target, n (%) | *449* | 239 (53) | 95 (58) | 144 (50) | 0.11 |
| LDL cholesterol ≤2.5 mmol/l, n (%) | *428* | 334 (78) | 127 (80) | 207 (77) | 0.53 |
| Serum HbA1c, % | *450* | 7.4 ± 3.2 | 6.4 ± 2.6 | 8.0 ± 3.1 | <0.001 |
| Serum HbA1c (mmol/mol) | *450* | 57 ± 12 | 46 ± 5 | 64 ± 10 | <0.001 |
| Glycosuria, g/24h | *361* | 0.5 [0.1-5.5] | 0.1 [0.0-0.4] | 2.0 [0.2-9.0] | <0.001 |
| *Co-morbidity* | | | | | |
| Microvascular disease, n (%) | *444* | 296 (67) | 104 (65) | 192 (68) | 0.46 |
| Nephropathy, n (%) | *446* | 189 (42) | 77 (48) | 112 (39) | 0.08 |
| eGFR§ <60, n (%) | *450* | 104 (23) | 49 (30) | 55 (19) | 0.008 |
| Albuminuria, n (%) | *445* | 136 (31) | 48 (30) | 88 (31) | 0.85 |
| Retinopathy, n (%) | *447* | 108 (25) | 26 (16) | 84 (30) | 0.002 |
| Neuropathy, n (%) | *450* | 162 (36) | 57 (35) | 105 (37) | 0.73 |
| Macrovascular disease, n (%) | *450* | 160 (36) | 64 (39) | 96 (33) | 0.22 |
| Coronary artery disease, n (%) | *450* | 100 (22) | 37 (23) | 63 (22) | 0.85 |
| Cerebrovascular disease, n (%) | *450* | 49 (11) | 20 (12) | 29 (10) | 0.48 |
| Peripheral artery disease, n (%) | *450* | 40 (9) | 18 (11) | 22 (8) | 0.23 |

**Table 1.** Continued

| *Variable* | *n* | *Total population* | *HbA1c-OIT* <7% (<53mmol/mol) | *HbA1c-NOIT* ≥7% (≥53 mmol/mol) | *P-value* |
|---|---|---|---|---|---|
| *Pharmacological management* | | | | | |
| Metformin, n (%) | *450* | 333 (74) | 120 (74) | 213 (74) | 0.89 |
| Sulfonylureas, n (%) | *450* | 114 (25) | 42 (26) | 72 (25) | 0.87 |
| DPP-4 inhibitors, n (%) | *450* | 19 (4) | 8 (5) | 11 (4) | 0.59 |
| GLP-1 analogues, n (%) | *450* | 45 (10) | 17 (10) | 28 (10) | 0.82 |
| SGLT-2 inhibitors, n (%) | *450* | 4 (1) | 0 (0) | 4 (1) | 0.13 |
| *Non-insulin users, n (%)* | *450* | 165 (37) | 97 (60) | 68 (24) | |
| *Number of used non-insulin agents* | *165* | | | | <0.001 |
| 0, n (% of non-insulin users) | *165* | 19 (12) | 17 (18) | 2 (3) | |
| 1, n (% of non-insulin users) | *165* | 57 (35) | 40 (41) | 17 (25) | |
| 2, n (% of non-insulin users) | *165* | 41 (25) | 22 (23) | 19 (28) | |
| 3, n (% of non-insulin users) | *165* | 18 (11) | 6 (6) | 12 (18) | |
| 4, n (% of non-insulin users) | *165* | 30 (18) | 12 (12) | 18 (27) | |
| *Insulin users, n (%)* | *450* | 285 (63) | 66 (41) | 219 (76) | <0.001 |
| Basal regimen, n (% of insulin users) | *285* | 36 (13) | 9 (14) | 27 (12) | 0.65 |
| Basal bolus/plus regimen, n (% of insulin users) | *285* | 160 (56) | 39 (59) | 121 (55) | |
| Mix regimen, n (% of insulin users) | *285* | 60 (21) | 14 (21) | 46 (21) | |
| Bolus only regimen, n (% of insulin users) | 285 | 29 (10) | 4 (6) | 25 (11) | |
| Total daily units of insulin, units/day | *285* | 82 ± 52 | 70 ± 42 | 86 ± 54 | 0.02 |
| Total daily units of insulin per kg body weight, units/kg | 285 | 0.83 ± 0.48 | 0.73 ± 0.39 | 0.88 ± 0.50 | 0.04 |
| *Dietary intake* | | | | | |
| Total energy intake, kcal/day | *439* | 1910 ± 644 | 1845 ± 617 | 1947 ± 658 | 0.12 |
| Intake of fibers, g/day | *439* | 21 ± 7 | 20 ± 7 | 21 ± 7 | 0.22 |
| Intake of carbohydrates, g/day | *439* | 206 ± 71 | 200 ± 68 | 209 ± 72 | 0.20 |
| *Carbohydrate intake from food groups* | | | | | |
| Bread, g carbohydrates/day | *439* | 59 [42-73] | 53 [41-72] | 61 [43-75] | 0.19 |
| Snacks, g carbohydrates/day | *439* | 24 [12-37] | 21 [9-34] | 26 [14-37] | 0.03 |
| Potatoes, g carbohydrates/day | *439* | 20 [12-30] | 20 [12-31] | 20 [12-30] | 0.93 |
| Dairy, g carbohydrates/day | *439* | 19 [12-29] | 19 [11-28] | 19 [13-29] | 0.51 |
| Fruit, g carbohydrates/day | *439* | 19 [10-29] | 16 [9-27] | 21 [11-31] | 0.12 |
| Rice/pasta/dough, g carbohydrates/day | *439* | 8 [4-14] | 7 [3-12] | 8 [4-15] | 0.09 |
| *Lifestyle guideline adherence* | | | | | |
| BMI ≤25 kg/m$^2$, n (%) | *448* | 24 (5) | 8 (5) | 16 (6) | 0.75 |
| Current smokers, n (%) | *450* | 75 (17) | 31 (19) | 44 (15) | 0.29 |
| Physical activity, n (%) | *433* | 253 (58) | 96 (60) | 157 (57) | 0.53 |

**Table 1.** Continued

| *Variable* | | *Total population* | *HbA1c-OIT* | *HbA1c-NOIT* | *P-value* |
|---|---|---|---|---|---|
| | *n* | | *<7% (<53mmol/mol)* | *≥7% (≥53 mmol/mol)* | |
| Vegetable intake, n (%) | *440* | 31 (7) | 11 (7) | 20 (7) | 0.92 |
| Fruit intake, n (%) | *440* | 122 (28) | 44 (28) | 78 (28) | 0.94 |
| Legume intake, n (%) | *440* | 257 (58) | 88 (55) | 169 (60) | 0.27 |
| Nuts intake, n (%) | *440* | 61 (14) | 13 (8) | 48 (17) | 0.008 |
| Fish intake, n (%) | *440* | 161 (37) | 56 (35) | 105 (38) | 0.60 |
| Fats and oils intake, n (%) | *440* | 286 (65) | 112 (70) | 174 (62) | 0.10 |
| Dairy intake, n (%) | *440* | 88 (20) | 29 (18) | 59 (21) | 0.46 |
| Red meat intake, n (%) | *440* | 54 (12) | 20 (13) | 34 (12) | 0.91 |
| Processed meat intake, n (%) | *440* | 8 (2) | 3 (2) | 5 (2) | 0.95 |
| Tea intake, n (%) | *440* | 36 (8) | 17 (11) | 19 (7) | 0.16 |
| Sweet beverages intake, n (%) | *440* | 150 (34) | 54 (34) | 96 (34) | 0.91 |
| Alcohol intake, n (%) | *438* | 310 (71) | 113 (71) | 197 (71) | 0.92 |
| Salt intake, n (%) | *443* | 53 (12) | 30 (19) | 23 (8) | 0.001 |

HbA1c, glycated hemoglobin; OIT, on ideal target; NOIT, not on ideal target; eGFR, estimated glomerular filtration rate; DPP4, Dipeptidylpeptidase-4; GLP-1, Glucagon-like peptide-1, SGLT-2, Sodium-glucose co-transporter-2.

Mean HbA1c in our population was 7.4% ± 3.2% (57 ± 12 mmol/mol). In total, 161 patients (36%) achieved an ideal HbA1c of <7% (<53 mmol/mol, HbA1c-OIT ), of which 33 patients (7% of total population) achieved a HbA1c <6% (<42 mmol/mol). There were no differences in age, gender and BMI between the HbA1c-OIT and HbA1c-NOIT groups. Patients in the latter group had a longer median duration of type 2 diabetes than those with HbA1c-OIT (13 [8-20] vs 9 [4-14] years, P<0.001), and a higher amount of glycosuria (2.0 [0.2-9.0] vs 0.1 [0.0-0.4] g/24h respectively, P<0.001). Also, patients with HbA1c-NOIT less often had an eGFR <60 ml/min (19% vs 30%, P=0.008), and more often had retinopathy (30% vs 15%, P=0.002).

Personalized HbA1c targets were achieved by 273 (61%) of patients (Supplementary Table S3). In general, patients with a more stringent personalized HbA1c target (<7%; <53 mmol/mol), less often achieved the target than those with the more liberal personalized targets: 42% achievement in the age group 45-64 without complications, and 72% in those with complications in the same age group (target <7.5%; <58 mmol/mol); 60% in the group ≥65 years without complications, and 71% in those aged ≥65 years with complications (target <8%; <64 mmol/mol).

*Pharmacological management and ideal HbA1c target achievement*
In the total population, 63% of patients used insulin, and insulin use was substantially higher in those with HbA1c-NOIT (76%) than in those with HbA1c-OIT (41%, P<0.001; Table 1). Overall, use of non-insulin blood glucose lowering drugs was similar between the groups. However, non-insulin users with HbA1c-NOIT used more non-insulin blood glucose lowering drugs per day than the non-insulin users with HbA1c-OIT (45% vs 18% used 3-4 drugs/day, P<0.001). In insulin users, the majority of patients were on a basal bolus/plus regimen (56%), and there were no differences in insulin regimens between the ideal HbA1c target groups. The amount of total daily units of insulin was significantly higher in those with HbA1c-NOIT than those with HbA1c-OIT (86 ± 54 vs 70 ± 42 units/day, P=0.02).

*Lifestyle management and ideal HbA1c target achievement*
Total energy intake was 1910 ± 644 kcal/day, and energy intake was somewhat higher in those with HbA1c-NOIT, 1947 ± 658 vs 1845 ± 617 kcal/day, although not statistically significant (P=0.12, Table 1). Total carbohydrate intake was 206 ± 71 g/day, and was non-statistically significantly higher in HbA1c-NOIT patients as well (209 ± 72 vs 200 ± 68 g/day, P=0.20).

In terms of dietary food sources of carbohydrate intake, the most important contributors were: bread 59 [42-73] g/day, snacks 24 [12-37] g/day, potatoes 20 [12-30] g/day, dairy 19 [12-29] g/day, fruit 19 [10-29] g/day and rice/pasta/dough 8 [4-14] g/day. Intake of carbohydrates from different food sources was mostly similar between the HbA1c groups, except for carbohydrate intake from snacks, which was significantly higher in HbA1c-NOIT patients (26 [14-37] vs 21 [9-34] g carbohydrates/day, P=0.03).

In general, adherence to Dutch lifestyle guidelines was low, and was mostly similar between the groups (Table 1). Patients with HbA1c-NOIT less often adhered to the guideline of dietary salt intake than those with HbA1c-OIT (8% vs 19%, P=0.001), and more often adhered to the guideline on nuts intake (17% vs 8%, P=0.008).

*Factors associated with intensity of glucose lowering treatment*
As we found that patients with HbA1c-NOIT had higher intensity blood glucose lowering therapy, we investigated factors associated with higher intensity of blood glucose lowering treatment. To this end, we divided patients in four groups: one group on only non-insulin treatment, and the other groups based on tertile of daily used insulin dosage (Table 2). HbA1c was higher in each higher group of treatment intensity (P<0.001), and thus lower ideal HbA1c target achievement per group (P<0.001), illustrating more treatment resistance along with a higher intensity of pharmacological treatment. Body mass

index was higher in every higher tertile of daily used insulin, tertile 1, 29.8 ± 5.5 kg/m$^2$; tertile 2, 31.9 ± 4.8 kg/m$^2$; tertile 3, 35.8 ± 5.5 kg/m$^2$ (P<0.001), which was paralleled by a higher waist/hip ratio in each higher tertile of insulin use (P<0.001). There was a non-statistically significant trend of higher daily energy intake in each higher group of treatment intensity (no insulin: 1762 [1388-2176] vs insulin tertile 3: 1969 [1548-2334] kcal/day, P=0.12) Additionally, total carbohydrate intake was higher in insulin users as compared to the non-insulin users (207 [168-256] vs 189 [149-234] g/day, P=0.03), while proteins and fat intake were not statistically different between the groups (P=0.09 and P=0.20 respectively). When assessing dietary sources of the higher carbohydrate intake, we found that carbohydrate intake derived from bread (62 [46-76] vs 53 [35-72] g/day, P=0.004), potatoes (21 [15-30] vs 18 [11-29] g/day, P=0.05), fruit (22 [13-31] vs 15 [8-27] g/day, P=0.002) and dairy (21 [13-31] vs 17 [10-26] g/day, P=0.007) were higher in insulin users (figure 1). Adherence to the Dutch Healthy Diet guidelines was mostly similar between all four groups (Data not shown). When analyzing factors associated with blood glucose lowering treatment intensity in HbA1c-OIT and HbA1c-NOIT patients separately, results were similar as in the current analysis (data not shown).

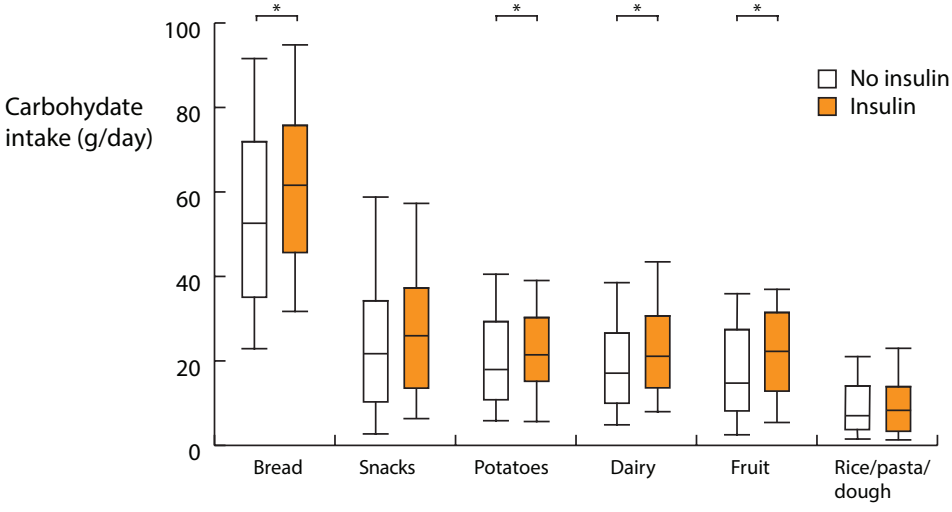


**Figure 1**. Boxplots of carbohydrate intake from different food sources in type 2 diabetes patients, by a breakup of insulin use. The brackets represents intake of the 10$^{th}$ and 90$^{th}$ percentile.
* significant difference (p<0.05) between non-insulin users and insulin users (Mann Whitney U).

**Table 2**. Patient characteristics by a breakup of blood glucose lowering treatment intensity

| *Variables* | *No insulin* |
|---|---|
| Insulin use, IE min-max | - |
| *Number of patients, n (% of total population)* | *166 (37)* |
| Total daily units of insulin, units/day | - |
| Total daily units of insulin per kg body weight, units/kg | - |
| Age, years | 62 ± 9 |
| Men, n (%) | 93 (56) |
| Diabetes duration, years | 7 [3-12] |
| Serum HbA1c, % | 6.9 ± 3.1 |
| Serum HbA1c, mmol/mol | 52 ± 10 |
| HbA1c <7% (<53 mmol/mol), n (%) | 97 (58) |
| Microvascular disease, n (%) | 90 (55) |
| Macrovascular disease, n (%) | 55 (33) |
| BMI, kg/m$^2$ | 33.5 ± 6.8 |
| Waist/hip ratio | 1.00 ± 0.09 |
| Adherent to guideline physical activity, n (%) | 91 (57) |
| *Dietary intake* | |
| Total energy intake, kilocalories/day | 1762 [1388-2176] |
| Urinary sodium excretion, mmol/day | 178 ± 78 |
| Urinary potassium excretion, mmol/day | 74 ± 24 |
| Sodium-to-potassium ratio, mmol/mmol | 2.51 ± 0.99 |
| Intake of protein, g/day | 73 [59-89] |
| Intake of fat, g/day | 71 [49-91] |
| Intake of carbohydrates, g/day | 191 [150-234] |
| *Carbohydrate intake from food groups* | |
| Bread, g carbohydrates/day | 53 [35-72] |
| Snacks, g carbohydrates/day | 22 [10-35] |
| Potatoes, g carbohydrates/day | 18 [11-29] |
| Dairy, g carbohydrates/day | 17 [10-26] |
| Fruit, g carbohydrates/day | 15 [8-27] |
| Rice/pasta/dough, g carbohydrates/day | 7 [4-14] |

HbA1c, glycated hemoglobin; BMI, body mass index

| *Insulin tertile 1* | *Insulin tertile 2* | *Insulin tertile 3* | *p-value* |
|---|---|---|---|
| 7-54 | 56-90 | 91-328 | |
| *93 (21)* | *96 (21)* | *95 (21)* | |
| 38 [28-44] | 70 [62-78] | 124 [106-163] | <0.001 |
| 0.41 ± 0.15 | 0.78 ± 0.16 | 1.31 ± 0.50 | <0.001 |
| 63 ± 9 | 64 ± 9 | 63 ± 8 | 0.25 |
| 56 (60) | 47 (49) | 65 (68) | 0.05 |
| 11 [7-17] | 15 [10-23] | 15 [11-20] | <0.001 |
| 7.5 ± 3.2 | 7.6 ± 3.2 | 7.8 ± 3.2 | <0.001 |
| 59 ± 12 | 60 ± 11 | 62 ± 11 | <0.001 |
| 26 (28) | 22 (23) | 16 (17) | <0.001 |
| 61 (67) | 67 (70) | 78 (83) | <0.001 |
| 31 (33) | 31 (32) | 43 (45) | 0.17 |
| 29.8 ± 5.5 | 31.9 ± 4.8 | 35.8 ± 5.5 | <0.001 |
| 0.98 ± 0.09 | 0.99 ± 0.08 | 1.04 ± 0.10 | <0.001 |
| 58 (63) | 48 (63) | 46 (50) | 0.11 |
| | | | |
| 1859 [1476-2293] | 1886 [1520-2318] | 1969 [1548-2334] | 0.12 |
| 178 ± 75 | 177 ± 73 | 218 ± 87 | <0.001 |
| 80 ± 27 | 77 ± 26 | 82 ± 25 | 0.07 |
| 2.34 ± 0.90 | 2.39 ± 0.87 | 2.77 ± 1.14 | 0.01 |
| 76 [67-91] | 77 [65-92] | 80 [67-97] | 0.09 |
| 73 [50-90] | 73 [59-93] | 78 [60-106] | 0.20 |
| 206 [155-243] | 208 [169-269] | 205 [174-260] | 0.03 |
| | | | |
| 61 [44-78] | 61 [46-73] | 63 [46-75] | 0.04 |
| 25 [9-34] | 25 [15-38] | 26 [15-41] | 0.11 |
| 20 [11-29] | 24 [17-30] | 21 [15-33] | 0.06 |
| 21 [14-33] | 20 [12-28] | 21 [14-34] | 0.05 |
| 20 [11-31] | 20 [13-33] | 24 [14-31] | 0.01 |
| 9 [4-14] | 8 [4-14] | 8 [3-14] | 0.65 |

# Discussion

We studied the prevalence of ideal HbA1c target achievement in a real-world setting of type 2 diabetes mellitus treatment, and aimed to pinpoint opportunities for improving target achievement . In this secondary care setting, the ideal target of <7% (53 mmol/mol) was not reached in roughly two-thirds of patients (64%), which is somewhat higher than the reported worldwide pooled-average (57%)[12]. The latter report, however, also included less complicated T2DM populations. Type 2 diabetes is a progressive disease, with decreasing endogenic insulin production over the years, which, along with insulin resistance, renders tight glycemic control in long-standing diabetes more challenging[24,25]. In our population, median diabetes duration was 11 years, and those not on the ideal HbA1c target had a longer duration of diabetes than those with HbA1c-OIT (median 13 vs 8 years). Evaluation of pharmacological treatment showed a high degree of treatment resistance, as patients who did not achieve the target more frequently used insulin (76%), and had a quite high average daily dose of insulin (86 units/day). Also, higher daily insulin dosage was paralleled by a higher body mass index. Therefore, the overall picture in those not on ideal HbA1c target, is that of a group on high intensity blood glucose lowering treatment caught in a vicious circle of increased insulin resistance, insulin use and obesity.

Because firm evidence for an advantage of the ideal HbA1c target of <7% (53 mmol/mol) is lacking in patients who are elderly and/or have extensive comorbidity, in such patients a more liberal HbA1c target may be accepted[11]. Of note, in the latter group, a more liberal HbA1c target is not the primary aim, but is accepted when the ideal target is not easily reached[11]. In our population, a relatively large proportion of patients fulfills the criteria for a more liberal HbA1c target. When we evaluated the extent to which such personalized HbA1c targets were achieved, we found, as expected, a considerably higher target achievement (61%). Because pharmacological treatment was more intensive in those who did not achieve the ideal HbA1c target, treatment resistance, and not the use of more liberal HbA1c targets per se, appears to be the major cause of not reaching the ideal target in this population.

In addition to treatment resistance, other factors could also play a role in low ideal target achievement. Pharmacological undertreatment was present in 57% of HbA1c-NOIT patients, as 24% was not on current insulin treatment, and 12% and 21% were on basal or mix insulin regimen. This indicates that there may be reasons not to start insulin, or start a basal bolus/plus regimen, such as patient preference, or inability to self-monitor blood glucose levels. In addition, treatment adherence should be addressed. Reports have found adherence rates of 20-50% for specific blood glucose lowering drug classes in type

2 diabetes patients[26,27], and low adherence has been associated with decreased HbA1c target achievement, along with worse clinical outcomes[28].

As a likely explanation for the therapy resistance, adherence to lifestyle guidelines was rather low in the studied population, and did not differ between those who did and did not achieve the ideal HbA1c target. With respect to the suboptimal dietary habits, this appears not specific for type 2 diabetes patients, since dietary intake of food groups in our population resembles that of the general Dutch population[17,29]. When looking at macronutrients, we found a median carbohydrate intake of 200 [157-246] g/day, which was lower than that of the Dutch general population (median intake 218 [183-256] g/day), and might be explained by lower intake of sweet beverages in DIALECT-1 (27 g/day in DIALECT-1 vs 336 g/day in the general population[17,30]. It has been well established that adopting a healthy lifestyle can improve glycemic control. A recent network meta-analysis demonstrated that adopting a healthy diet, regardless of dietary approach, significantly reduced HbA1c (-0.82 to -0.47%) and fasting glucose (-1.61 to -1.00 mmol/l), where HbA1c lowering was most pronounced in low-carbohydrate diets[31]. This was in line with our finding that patients not on insulin had a lower dietary carbohydrate intake than those on insulin. In addition, increasing physical activity may improve insulin sensitivity in type 2 diabetes patients; aerobic and resistance training can improve insulin action[32], and structured exercise training has been associated with a HbA1c decline of -0.67% (95% confidence interval -0.84% to -0.49%)[33]. Lastly, weight loss can considerably lower HbA1c, and can even lead to a remission of type 2 diabetes, also in patients already on insulin[34,35].

The main strengths of this study are the real-world data and the integrated analysis of both lifestyle and pharmacological management. A limitation of this study is possible reverse causality bias, due to the cross-sectional setting. In addition, Also, the use of the food frequency questionnaires to assess diet might lead to underestimation of intake of unhealthy products in this obese population, leading to an underrepresentation of total energy intake[36]. Nevertheless, there are currently no better methods for registration of dietary habits in a study with this size. Of note, even if unhealthy products were underreported, our study reveals plenty of opportunities for improving dietary habits. Lastly, a limitation is that in our study, treatment failure due to treatment resistance or treatment-non adherence could not be distinguished. However, in previous studies we found no signs of treatment non-adherence in this population, because patients not on target blood pressure and LDL cholesterol did not use higher intensity antihypertensive and lipid lowering therapy respectively[16,17].

The question rises how ideal HbA1c target achievement can be improved in clinical practice. In our opinion, given the apparent treatment resistance and insulin-resistance, the aim should be to improve insulin sensitivity, ideally by lifestyle intervention. The high degree of obesity, especially in those in the highest tertile of insulin use, marks the opportunity for weight loss in this population. Next to that, adherence to the Dutch Healthy Diet guidelines was poor in our population, in particular for vegetable intake, illustrating that patients have many options to improve the quality of their diet. Intensifying pharmacological therapy may also improve glycemic control. In patients not on insulin, or in on a basal or mix insulin regimen, insulin therapy could be initiated or switched to a basal bolus/plus regimen, but this is associated with weight gain and may fuel the vicious circle of insulin resistance. Moreover, increasing insulin dose appears to have limited efficacy: 17% of our population did not achieve the ideal HbA1c target despite 91+ units of insulin/day. n our opinion, pharmacological therapy should preferably be applied to support lifestyle intervention and be aimed to improve insulin resistance. As important options, glucagon-like peptide-1 (GLP-1) analogues and sodium-glucose co-transporter-2 (SGLT-2) inhibitors could be valuable in improving glycemic control, as they do not only reduce HbA1c, but also reduce weight and long term cardiovascular risk, without increased risk of hypoglycemia[37-39]. As these agents are available for regular type 2 diabetes care in the Netherlands since 2012 and 2014, and reimbursement is available since 2017 and 2016, use of these drugs was relatively low in our cohort, 10% and 1% respectively, because patients were included between 2009 and 2016.

Previously we determined achievement of other CVRM targets in this population, the blood pressure and LDL cholesterol targets were reached in half and three quarters of patients respectively[16,17]. For blood pressure, pharmacological undertreatment was present in 62% of those not on target, and for LDL cholesterol this was 92%, while for both targets lifestyle was suboptimal in 97% of patients not on target. Here we demonstrate that for HbA1c, pharmacological undertreatment is present in 57% of patients. However, treatment resistance is high in patients with high intensity blood glucose lowering pharmacological treatment, and therefore the best opportunity is for improving HbA1c target achievement, is improving insulin sensitivity through lifestyle intervention. We speculated that our integral data on opportunities in lifestyle and pharmacological treatment may provide a basis for shared decision making in CVRM in clinical practice.

## Conclusion

Ideal HbA1c target achievement is low in this real-life population of type 2 diabetes patients treated in secondary care. Pharmacological treatment resistance was high, and was paralleled by high BMI. Therefore, treatment should be aimed at increasing insulin sensitivity, through lifestyle interventions such as weight reduction, increasing physical activity and adopting a healthy diet.

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**Supplementary Table S1.** Overview of food items included in each food category to determined adherence to the Dutch Healthy Diet guideline.

| | |
|---|---|
| 1 | **Vegetables** |
| | Broccoli, cauliflower etc. |
| | Sal, spinach, endive etc. |
| | Mushrooms |
| | Onion, pepper, etc |
| | Raw vegetables |
| 2 | **Fruit** |
| | Citrus fruit |
| | Other fruit |
| 3 | **Legumes** |
| | Legumes |
| | Split peas, beans etc. |
| | Split pea soup |
| 4 | **Nuts** |
| | Nuts and seeds |
| | Peanuts (and coated peanuts) |
| | Nuts as in between snack |
| 5 | **Dairy** |
| | Semi-skimmed milk |
| | Skimmed milk |
| | Buttermilk |
| | Other milk, such as horse and goat |
| | Normal milk in coffee |
| | Semi-skimmed milk beverages |
| | Skimmed milk beverages |
| | Semi-skimmed (fruit) yogurt |
| | Skimmed (fruit) yogurt |
| | Cholesterol-lowering yoghurt drink |
| | Low-fat custard & pudding |
| | Lean custard & pudding |
| | 20+/30+ cheese |
| | 40+ cheese |
| | Semi-skimmed (fruit) cream cheese |
| | Skimmed (fruit) cream cheese |
| | Hot porridge |
| 6 | **Fish** |
| | Flounder, etc |
| | Unknown type of fish |
| | Herring |
| | Diet liquid margarine |
| | Liquid baking fats |
| | Vegetable oils |
| | Diet marganine |
| | Stanols margarine |
| | Liquid margarine |
| | Diet liquid margarine |
| | Liquid baking fats |
| | Vegetable oils |
| 8 | **Hard margarines** |
| | Butter |
| | Low-fat butter |
| | Solid margarine |
| | Solid baking fats |
| | Lard |
| 9 | **White meat** |
| | Chicken, turkey |
| 10 | **Red meat** |
| | Beef, steak etc. |
| | Beef, blind finch, etc. |
| | Pork, steak etc. |
| | Pork, chop etc. |
| | Pork, smoked sausage etc. |
| | Lamb or mutton |
| | Other meats such as goat/horse |
| | Cooked liver |
| | Hepatic/renal products |
| | Sausages as in between snack |
| | Minced meat (all types) |
| | Liver products |
| | Ham etc. |
| | Lunch meats |
| | Bacon etc. |
| | Unknown type of meat |
| 11 | **Processed meat** |
| | Beef, blind finch, etc. |
| | Pork, steak etc. |
| | Pork, smoked sausage etc. |
| | Sausages as in between snack |

**Supplementary Table S1.** Continued.

| | |
|---|---|
| | Salmon, etc |
| | Shellfish |
| | Trout, etc |
| 7 | **Soft margarines/oils** |
| | Oil salad dressing |
| | Halvarine |
| | Diet halvarine |
| | Stanols halvarine |
| | Margarine |
| | Diet marganine |
| | Stanols margarine |
| | Liquid margarine |
| | Minced meat (all types) |
| | Liver products |
| | Ham etc. |
| | Lunch meats |
| | Bacon etc. |
| 12 | **Tea** |
| | Tea |
| 13 | **Sweet beverages** |
| | Fruit drinks |
| | Soda |
| | Sports drinks |

**Supplementary Table S2.** Overview of food items included in each carbohydrate dense food category to calculate dietary origin of carbohydrate intake.

| | |
|---|---|
| 1 | **Bread, total** |
| | Bread |
| | Rye bread |
| | Raisin bread |
| | Rusk |
| | Croissants |
| | Bread rolls |
| 2 | **Dairy** |
| | Milk, full |
| | Milk, semi-skimmed |
| | Milk, skimmed |
| | Buttermilk |
| | Milk, other such as horse and goat |
| | Milk, soy based |
| | Milk, rice or oat based |
| | Milk, unknown types |
| | Milk, normal in coffee |
| | Milk, coffee cream |
| | Milk, coffee milk powder |
| | Drinks, full |
| | Drinks, semi-skimmed |
| | Drinks, skimmed |
| | Drinks, soy based |
| | Drinks, unknown dairy based |
| 3 | **Diner total (dough and rice based)** |
| | Pizzas |
| | Pancakes |
| | Pastas |
| | Ready-made meals, Italian |
| | Ready-made meals, savoury cakes |
| | Ready-made meals, other |
| | Rice |
| 4 | **Potatoes** |
| | Potatoes, cooked/mashed |
| | Potatoes, baked |
| | Fries, in oven |
| | Fries, self-made |
| | Fries, self-fried |
| | Fries, ready-made |
| 5 | **Fruit** |
| | Citrus fruit |
| | Other fruit |
| | Canned fruit |
| 6 | **Snacks** |
| | Cookies, small |
| | Cookies, big and cake |
| | Nutrition biscuit |
| | Pie and pastries |

**Supplementary Table S2.** Continued

| | |
|---|---|
| Drinks, cholesterol lowering | Candy, bars |
| Drinks, blood pressure lowering | Candy, chocolates and chocolate bars |
| Beverages with probiotics | Candy, drop |
| Yogurt, full (fruits) | Candy, other |
| Yogurt, semi-skimmed (fruits) | Snacks, meat |
| Yogurt, skimmed (fruits) | Snacks, small with meat |
| Yogurt, with probiotics | Snacks, cheese |
| Yogurt, soy based | Snacks, small with cheese |
| Custard and pudding, full | Snacks, satay with sauce |
| Custard and pudding, semi-skimmed | Snacks, small spring roll |
| Custard and pudding, skimmed | Snacks, large spring roll |
| Cottage cheese, full (fruits) | Snacks, ready-made baked fish |
| Cottage cheese, semi-skimmed (fruits) | Snacks, salad |
| Cottage cheese, skimmed (fruits | Snacks, chips and salts |
| Deserts, soy based | Snacks, toast with toppings |
| Ice cream, cream | |
| Ice cream, water based | |
| Ice cream, soy based | |
| Whipped cream | |

**Supplementary Table S3.** Patient characteristics of DIALECT-1 by a breakup of personalized HbA1c target achievement.

| Variable | | Personalized HbA1c-OT | Personalized HbA1c-NOT | P-value |
|---|---|---|---|---|
| ***Number of patients, n (%)*** | *Target* | *n=273 (61)* | *n=172 (39)* | |
| *Age <45, complications* | *<53 mmol/mol* | 1 (25) | 3 (75) | |
| *Age 45-64, no complications* | *<53 mmol/mol* | 41 (42) | 56 (58) | |
| *Age 45-64, complications* | *<58 mmol/mol* | 83 (72) | 32 (28) | |
| *Age ≥65, no complications* | *<53 mmol/mol* | 45 (60) | 30 (40) | |
| *Age ≥65, complications* | *<64 mmol/mol* | 99 (71) | 41 (29) | |
| Age, years | | 64 ± 8 | 62 ± 10 | 0.01 |
| Men, n (%) | | 166 (61) | 93 (54) | 0.16 |
| Diabetes duration, years | | 11 [5-17] | 12 [8-20] | 0.004 |
| Body mass index, kg/m$^2$ | | 32.4 ± 6.3 | 33.5 ± 6.1 | 0.06 |
| Waist/hip ratio, cm/cm | | 1.01 ± 0.09 | 1.00 ± 0.10 | 0.68 |
| Systolic blood pressure, mmHg | | 137 ± 17 | 135 ± 15 | 0.25 |
| Diastolic blood pressure, mmHg | | 74 ± 10 | 75 ± 9 | 0.83 |
| Heart frequency, beats/min | | 73 ± 13 | 76 ± 12 | 0.02 |
| Blood pressure on target, n (%) | | 92 (54) | 142 (53) | 0.80 |
| LDL cholesterol ≤2.5 mmol/l, n (%) | | 126 (77) | 204 (79) | 0.67 |

**Supplementary Table S3.** Continued.

| Variable | | Personalized HbA1c-OT | Personalized HbA1c-NOT | P-value |
|---|---|---|---|---|
| ***Number of patients, n (%)*** | *Target* | *n=273 (61)* | *n=172 (39)* | |
| Serum HbA1c, % | | 6.8 ± 2.8 | 8.4 ± 3.1 | <0.001 |
| Serum HbA1c, mmol/mol | | 51 ± 7 | 68 ± 10 | <0.001 |
| Glycosuria, g/24h | | 0.2 [0.1-1.6] | 3.5 [0.3-9.6] | <0.001 |
| *Co-morbidity* | | | | |
| Microvascular disease, n (%) | | 196 (72) | 99 (58) | 0.001 |
| Nephropathy, n (%) | | 135 (50) | 52 (30) | <0.001 |
| eGFR <60, n (%) | | 75 (28) | 27 (16) | 0.004 |
| Albuminuria, n (%) | | 98 (36) | 37 (22) | 0.001 |
| Retinopathy, n (%) | | 69 (25) | 40 (23) | 0.61 |
| Neuropathy, n (%) | | 102 (37) | 59 (34) | 0.51 |
| Macrovascular disease, n (%) | | 114 (42) | 46 (27) | 0.001 |
| Coronary artery disease, n (%) | | 71 (26) | 29 (17) | 0.02 |
| Cerebrovascular disease, n (%) | | 37 (14) | 12 (7) | 0.03 |
| Peripheral artery disease, n (%) | | 27 (10) | 12 (7) | 0.29 |
| *Pharmacological management* | | | | |
| Metformin, n (%) | | 197 (72) | 132 (77) | 0.28 |
| Sulfonylureas, n (%) | | 67 (25) | 44 (26) | 0.81 |
| DPP-4 inhibitors, n (%) | | 13 (5) | 6 (4) | 0.52 |
| GLP-1 receptor agonists, n (%) | | 22 (8) | 23 (13) | 0.07 |
| SGLT2-inhibitors, n (%) | | 1 (0) | 2 (1) | 0.32 |
| *Non-insulin users, n (%)* | | 122 (45) | 40 (23) | <0.001 |
| *Number of used non-insulin agents* | | | | 0.001 |
| 0, n (%) | | 18 (15) | 1 (3) | |
| 1, n (%) | | 48 (39) | 8 (20) | |
| 2, n (%) | | 31 (25) | 10 (25) | |
| 3, n (%) | | 11 (9) | 7 (18) | |
| 4, n (%) | | 15 (12) | 14 (35) | |
| *Insulin users, n (%)* | | 150 (55) | 132 (77) | <0.001 |
| Basal regimen, n(%) | | 19 (13) | 17 (13) | 0.89 |
| Basal bolus/plus regimen, n (%) | | 83 (55) | 76 (58) | |
| Mix regimen, n (%) | | 34 (23) | 25 (19) | |
| Total daily units of insulin, units/day | | 79 ± 53 | 87 ± 50 | 0.22 |
| *Dietary intake* | | | | |
| Total energy intake, kcal/day | | 1878 ± 647 | 1961 ± 637 | 0.19 |
| Intake of fibers, g/day | | 20 ± 7 | 22 ± 7 | 0.06 |

**Supplementary Table S3.** Continued.

| Variable | | Personalized HbA1c-OT | Personalized HbA1c-NOT | P-value |
|---|---|---|---|---|
| ***Number of patients, n (%)*** | *Target* | *n=273 (61)* | *n=172 (39)* | |
| Intake of carbohydrates, g/day | | 203 ± 70 | 210 ± 71 | 0.32 |
| *Carbohydrate intake from food groups* | | | | |
| Bread, g carbohydrates/day | | 55 [42-73] | 62 [41-76] | 0.31 |
| Snacks, g carbohydrates/day | | 22 [12-34] | 26 [15-38] | 0.08 |
| Potatoes, g carbohydrate/day | | 20 [12-29] | 22 [12-30] | 0.26 |
| Dairy, g carbohydrates/day | | 18 [11-28] | 21 [14-30] | 0.15 |
| Fruit, g carbohydrates/day | | 16 [10-31] | 21 [12-29] | 0.14 |
| Rice/pasta/dough, g carbohydrates/day | | 7 [3-13] | 9 [5-15] | 0.02 |
| *Lifestyle guideline adherence* | | | | |
| BMI ≤25 kg/m$^2$, n(%) | | 15 (6) | 9 (5) | 0.89 |
| Non-smokers, n (%) | | 221 (83) | 147 (86) | 0.14 |
| Physical activity, n (%) | | 166 (63) | 86 (52) | 0.03 |
| Vegetable intake, n (%) | | 14 (5) | 17 (10) | 0.05 |
| Fruit intake, n (%) | | 70 (26) | 50 (30) | 0.39 |
| Legume intake, n (%) | | 149 (56) | 105 (63) | 0.13 |
| Nuts intake, n (%) | | 31 (12) | 29 (17) | 0.09 |
| Fish intake, n (%) | | 95 (35) | 62 (37) | 0.72 |
| Fats and oils intake, n (%) | | 184 (69) | 100 (60) | 0.06 |
| Dairy intake, n (%) | | 49 (18) | 38 (23) | 0.26 |
| Red meat intake, n (%) | | 36 (13) | 17 (10) | 0.31 |
| Processed meat intake, n (%) | | 4 (2) | 4 (2) | 0.50 |
| Tea intake, n (%) | | 25 (9) | 11 (7) | 0.31 |
| Sweet beverages intake, n (%) | | 93 (35) | 57 (34) | 0.90 |
| Alcohol intake, n (%) | | 184 (69) | 122 (74) | 0.29 |
| Salt intake, n (%) | | 37 (14) | 16 (9) | 0.17 |

HbA1c, glycated haemoglobin; OT, on target; NOT, not on target; eGFR, estimated glomerular filtration rate; DPP4, Dipeptidylpeptidase-4; GLP1, Glucagon-like peptide-1, SGLT2, Sodium-glucose co-transporter 2.

# CHAPTER 5

## Higher Dietary Magnesium Intake and Higher Magnesium Status are Associated with Lower Prevalence of Coronary Heart Disease

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# Abstract

**Background** In type 2 diabetes mellitus (T2D), handling of magnesium is disturbed. Magnesium deficiency may be associated with a higher risk of coronary heart disease (CHD). We investigated the associations between 1) dietary magnesium intake, 2) 24h urinary magnesium excretion and 3) plasma magnesium concentration with prevalent CHD in T2D patients.

**Methods** This cross-sectional analysis was performed on baseline data from the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1, *n*=450, age 63 ± 9 years, 57% men, diabetes duration 11 [7-18] years). Prevalence ratios (95% CI) of CHD by sex-specific quartiles of magnesium indicators, as well as by magnesium intake per dietary source, were determined using multivariable Cox proportional hazard models.

**Results** CHD was present in 100 (22%) subjects. Adjusted CHD prevalence ratios for the highest compared to the lowest quartiles were: 0.40 (0.20, 0.79) for magnesium intake, 0.63 (0.32, 1.26) for 24h urinary magnesium excretion and 0.62 (0.32, 1.20) for plasma magnesium concentration. For every 10mg increase of magnesium intake from vegetables, prevalence of CHD was, statistically non-significantly, lower (0.75 (0.52, 1.08)).

**Conclusion** In this T2D cohort, higher magnesium intake, higher 24h urinary magnesium excretion and higher plasma magnesium concentration, are associated with a lower prevalence of CHD.

# Introduction

Coronary heart disease (CHD) is one of the most prevalent and high-impact complications in type 2 diabetes mellitus (T2D)[1,2]. In the general population, magnesium (Mg) deficiency might be associated with a greater risk of CHD, however data on the inverse associations between Mg status and intake, and CHD are inconsistent[3-9]. In T2D, the prevalence of hypomagnesemia is increased, 14-48%, compared with 3-15% in those without T2D[10,11]. This could partly be due to increased urinary Mg excretion caused by insulin resistance, and partly due to poor dietary Mg intake[10-13]. However, surprisingly few studies report on the association between Mg and CHD in patients with established T2D[14,15].

In the DIAbetes and LifEstyle Cohort Twente (DIALECT) we collected extensive data on dietary Mg intake, 24h urinary Mg excretion and plasma Mg concentration. We aimed to study the association between parameters of Mg (i.e. dietary Mg intake, 24h urinary Mg excretion, serum Mg concentration) and CHD. When we found that dietary Mg intake was inversely associated with CHD risk, we also explored whether there was an association between Mg intake from specific dietary sources (i.e. cereals, potatoes, etc.) and CHD risk.

# Materials and Methods

*Study design*

This was a cross-sectional analysis performed in the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1). The study population and study procedures were described previously[16]. The study has been approved by the relevant institutional review boards (METC-Twente, NL57219.044.16; METC-Groningen, 1009.68020), is registered in the Netherlands Trial Register (NTR trial code 5855) and is performed according to the guidelines of good clinical practice and the declaration of Helsinki.

*Participants*

All patients with T2D treated in the outpatient clinic of our hospital, aged 18+ years, were eligible for the study. Exclusion criteria were inability to understand the informed consent procedure, insufficient command of the Dutch language, or dialysis dependency. The inclusion flowchart is described previously[16]. In total, 1082 patients were eligible for the study, of which 470 agreed to participation. The most important reasons for non-participation were: no interest in trial ($n$=123), inability due to co-morbidity ($n$=62) and no transport options ($n$=58). After the baseline visit, 20 patients were excluded due to the fact that their diabetes diagnosis changed from type 2 diabetes to type 1 diabetes. Therefore, in total 450 patients with type 2 diabetes were included in DIALECT-1. Missing values for specific variables are listed in Table 1.

*Study procedures*

Eligible patients with type 2 diabetes were selected from the electronic patient file. At the clinic, sociodemographic characteristics, medical history, lifestyle behaviors, and current medications were recorded and anthropometric dimension were measured. Blood pressure was measured in a supine position by an automated device (Dinamap®; GE Medical systems, Milwaukee, WI) for 15 minutes with a one-minute interval. The mean systolic and diastolic pressure of the final three measurements was used for further analysis. Physical activity was assessed using the Short QUestionnaire to ASses Health enhancing physical activity (SQUASH) questionnaire, which was previously validated and is commonly used in the Netherlands for population research[17].

Blood was drawn from venipuncture, for measurement of Mg and other variables relevant for diabetes. 24-hour urine collections were performed as prescribed previously[16]. Samples of blood and urine were stored at -80°C for later analysis.

*Magnesium measurements*

We calculated dietary Mg intake using a semi-quantitative food-frequency questionnaire (FFQ) inquiring about intake of 177 items during the last month, taking seasonal variations into account[18]. The FFQ was developed and validated at the Wageningen University and has been updated several times. For each item, the frequency was recorded in times per day, week, or month. The number of servings was expressed in natural units (e.g., slice of bread or apple) or household measures (e.g., cup or spoon). Both questionnaires were self-administered and filled in at home. The filled in questionnaires were checked for completeness by a trained researcher, and inconsistent answers were verified with the patients. If the patient could not remember an exact number, the trained researcher approximated the intake as closely as possible during the interview. Dietary data was converted into daily nutrient intake using the Dutch Food Composition Table of 2013[19]. We calculated the average intake of Mg by multiplying the frequency of consumption of each food by its Mg content in the Dutch Food Composition Table of 2013[19] and summing across all foods. We calculated Mg intake from different food categories by multiplying the frequency of consumption of each food item in that specific category by its Mg content and summing across all foods in that category. Food items of the FFQ included in each category are listed in supplementary Table 1.

Plasma and urinary Mg was measured in stored plasma samples in routine laboratory measurements using the xylidyl blue method. Buffer/EGTA was added to mask calcium. After incubation, xylidyl blue was added to form a purple complex with Mg. Mg concentration is determined by photometric measurement of xylidyl blue extinction. The detection range for plasma Mg was 0.1-5 mmol/l, and for 24h urinary Mg this was 0.5-25 mmol/l. There were no patients with values outside of the detection ranges. Hypomagnesemia was defined as serum Mg concentration <0.70 mmol/l.

*Main study outcome*

Coronary heart disease (CHD) was defined as physician diagnosed unstable angina pectoris or myocardial infarction, percutaneous coronary intervention or coronary artery bypass graft in the medical history. Medical history was checked for CHD during the interview at the baseline visit, and was later reviewed in the hospital electronic patient files on three different occasions, by three different physician researchers who were unware of the magnesium data.

*Statistics*

All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS), version 23.0. Normally distributed data are presented as mean ± standard deviation. Skewed variables are expressed as median [interquartile range]. Dichotomous variables are presented in number and percentage. Dietary intake of Mg was adjusted for energy intake by the residual method[20].

Differences between T2D patients with and without CHD were determined using Student t test (normal distribution), Mann Whitney U (skewed distribution) or Chi-Square (categorical variables). In order to examine parameters associated with magnesium intake, we divided the population in sex-specific quartiles of adjusted magnesium intake. Differences between the quartiles were assessed using one-way ANOVA (normal distribution), Kruskal Wallis (skewed distribution) or Chi Square (categorical variables). Correlations between Mg parameters were assessed using Pearson's correlation coefficient.

We calculated the prevalence ratio (95% CI) of CHD by sex-specific quartiles of: 1) dietary Mg intake, 2) 24h urinary Mg excretion and 3) plasma Mg concentration using multivariable Cox proportional hazard models, with the time to event set at 1 for all patients. The models were adjusted for potential confounding of lifestyle parameters (BMI, smoking, alcohol, physical activity) and nutritional intake (24h urinary sodium excretion and 24h urinary potassium excretion)[3,21]. There was a strong correlation between dietary calcium and Mg intake (R=0.70), therefore we did not adjust for calcium intake in the final model. Effect modification was checked for gender, BMI, smoking and alcohol, and no significant effect modification was found (P>0.20 for all interaction terms). Sensitivity analyses were performed by excluding patients with diabetic kidney disease, and prevalence ratios were similar as in the primary analyses.

Additionally, we performed multivariable Cox proportional hazard models and calculated the prevalence ratios of CHD for each 10mg increment of dietary Mg intake from different sources (cereals, dairy, coffee, potatoes, meat, legumes & nuts, fruit, vegetables and other). The models were adjusted for potential confounding of lifestyle parameters (age, BMI, smoking, alcohol, physical activity) and Mg intake from the miscellaneous sources.

# Results

In total, 450 patients with T2D were included in DIALECT-1. Baseline characteristics are shown in Table 1. In short, patients were 63 ± 9 years old, and the majority of the population was male (57%). The population represents T2D in secondary health care, with a median diabetes duration of 11 [7-18] years, and a high prevalence of diabetic nephropathy (42%).

**Table 1.** Baseline characteristics of patients with T2D by a breakup of prevalent coronary heart disease

| | | Total population | No CHD | CHD | P-value |
|---|---|---|---|---|---|
| | *n* | *n=450* | *n=350 (78%)* | *n=100 (22%)* | |
| *Patient characteristics* | | | | | |
| Age, years | *450* | 63 ± 9 | 62 ± 9 | 66 ± 7 | <0.001 |
| Men, n (%) | *450* | 261 (58) | 190 (54) | 71 (71) | 0.003 |
| Diabetes duration, years | *450* | 11 [7-18] | 11 [7-17] | 13 [7-20] | 0.15 |
| Systolic blood pressure, mmHg | *449* | 136 ± 16 | 136 ± 16 | 136 ± 19 | 0.81 |
| Diastolic blood pressure, mmHg | *449* | 74 ± 9 | 75 ± 9 | 72 ± 10 | 0.01 |
| Heart rate, beats/min | *444* | 74 ± 13 | 75 ± 13 | 69 ± 11 | <0.001 |
| Body surface area, $m^2$ | *448* | 2.10 ± 0.22 | 2.10 ± 0.23 | 2.07 ± 0.19 | 0.27 |
| Urinary creatinine excretion, µmol/24h | *446* | 13.8 ± 4.8 | 13.8 ± 5.0 | 13.8 ± 4.2 | 0.97 |
| *Complications* | | | | | |
| Cerebrovascular disease, n (%) | *450* | 47 (11) | 87 (22) | 13 (27) | 0.44 |
| Peripheral artery disease, n (%) | *450* | 44 (10) | 80 (20) | 20 (44) | <0.001 |
| Retinopathy, n (%) | *447* | 106 (24) | 78 (23) | 32 (32) | 0.05 |
| Neuropathy, n (%) | *450* | 157 (36) | 116 (33) | 46 (46) | 0.02 |
| Diabetic nephropathy, n (%) | *446* | 183 (42) | 131 (38) | 58 (58) | <0.001 |
| eGFR<60 | *450* | 101 (23) | 74 (21) | 30 (30) | 0.06 |
| Microalbuminuria, n (%) | *445* | 131 (30) | 92 (27) | 44 (44) | 0.001 |
| *Lifestyle* | | | | | |
| Body mass index, $kg/m^2$ | *448* | 32.8 ± 6.2 | 33.1 ± 6.4 | 32.1 ± 5.6 | 0.15 |
| Body mass index ≥ 30 $kg/m^2$, n (%) | *448* | 290 (65) | 233 (67) | 57 (58) | 0.12 |
| Smoking, former or current, n (%) | *450* | 306 (70) | 235 (67) | 78 (78) | 0.04 |
| Alcohol | *424* | | | | |
| No alcohol, n (%) | | 148 (36) | 123 (37) | 32 (34) | 0.80 |
| 0-13 units per week, n (%) | | 206 (50) | 159 (48) | 49 (52) | |
| 14+ units per week, n (%) | | 61 (15) | 47 (14) | 14 (15) | |
| Adherence guideline physical activity, n (%) | *433* | 249 (59) | 201 (60) | 52 (54) | 0.34 |

**Table 1.** Continued

| | n | Total population n=450 | No CHD n=350 (78%) | CHD n=100 (22%) | P-value |
|---|---|---|---|---|---|
| *Pharmacological treatment* | | | | | |
| Insulin use, n (%) | 450 | 275 (63) | 218 (62) | 68 (68) | 0.30 |
| Statin use, n (%) | 450 | 331 (76) | 254 (73) | 86 (86) | 0.006 |
| Beta blocker treatment, n (%) | 450 | 202 (46) | 131 (37) | 77 (77) | <0.001 |
| RAAS inhibition, n (%) | 450 | 289 (66) | 225 (64) | 73 (73) | 0.10 |
| Calcium antagonists, n (%) | 450 | 98 (22) | 66 (19) | 36 (36) | <0.001 |
| Thiazide diuretics, n (%) | 450 | 136 (31) | 108 (31) | 29 (29) | 0.72 |
| Loop diuretics, n (%) | 450 | 75 (17) | 48 (14) | 33 (33) | <0.001 |
| Number of antihypertensives | 450 | 2 [1-3] | 2 [1-3] | 3 [2-4] | <0.001 |
| *Magnesium parameters* | | | | | |
| Dietary magnesium intake*, mg/day | 438 | 305 ± 46 | 309 ± 47 | 292 ± 40 | 0.001 |
| Urinary magnesium excretion, mmol/24h | 402 | 3.94 ± 2.05 | 4.03 ± 2.05 | 3.66 ± 2.02 | 0.13 |
| Plasma magnesium concentration, mmol/l | 432 | 0.77 ± 0.09 | 0.78 ± 0.08 | 0.76 ± 0.09 | 0.06 |
| Hypomagnesemia, n (%) | 432 | 73 (17) | 53 (16) | 20 (20) | 0.35 |
| *Serum values* | | | | | |
| Total cholesterol, mmol/l | 447 | 4.0 ± 0.9 | 4.1 ± 0.9 | 3.8 ± 1.1 | 0.04 |
| HDL cholesterol, mmol/l | 445 | 1.1 ± 0.3 | 1.2 ± 0.4 | 1.0 ± 0.3 | <0.001 |
| LDL cholesterol, mmol/l | 428 | 2.0 ± 0.7 | 2.0 ± 0.7 | 1.9 ± 0.8 | 0.25 |
| HbA1c, mmol/mol | 448 | 57 ± 12 | 57 ± 12 | 58 ± 12 | 0.43 |
| *Dietary intake* | | | | | |
| Total energy intake, kcal/day | 438 | 1922 ± 629 | 1904 ± 649 | 1932 ± 630 | 0.71 |
| Urinary sodium excretion, mmol/24h | 444 | 185 ± 79 | 183 ± 67 | 197 ± 84 | 0.14 |
| Urinary potassium excretion, mmol/24h | 439 | 77 ± 25 | 78 ± 26 | 77 ± 21 | 0.87 |
| Calcium intake, mg/day | 438 | 969 ± 441 | 979 ± 467 | 905 ± 358 | 0.16 |
| Fiber intake, g/day | 438 | 20.9 ± 6.6 | 20.8 ± 7.0 | 20.4 ± 6.1 | 0.60 |
| Cholesterol, g/day | 438 | 194 ± 96 | 195 ± 101 | 188 ± 79 | 0.51 |
| Total fat intake, g/day | 438 | 79 ± 39 | 78 ± 34 | 81 ± 34 | 0.52 |
| Total protein intake, g/day | 438 | 79 ± 23 | 79 ± 24 | 76 ± 22 | 0.18 |
| Total carbohydrate intake, g/day | 438 | 207 ± 69 | 205 ± 72 | 209 ± 67 | 0.61 |

CHD coronary heart disease, eGFR estimated glomerular filtration rate (CKD-EPI), HDL high density lipoprotein, LDL low density lipoprotein, HbA1c glycated hemoglobin.* Dietary magnesium intake was adjusted for total energy intake using the residual method.

There were 100 (22%) CHD cases diagnosed in our population (Table 1). T2D patients with CHD were older (66 ± 7 vs 62 ± 9 years, P<0.001), more often were men (71% vs 54%, P=0.003), and more often had peripheral artery disease (44% vs 20%, P<0.001), and nephropathy (58% vs 38%, P<0.001) than those without CHD. There were no differences in lifestyle parameters between those with and without CHD. Regarding pharmacological

treatment, those with CHD more often used beta-blockers (77% vs 37%, P<0.001), and loop diuretics (33% vs 14%, P<0.001) than those without CHD. This was paralleled by a lower diastolic blood pressure (72 ± 10 vs 75 ± 9 mmHg, P=0.01) and heart rate (69 ± 11 vs 75 ± 13 beats/min, P<0.001) in CHD. Systolic blood pressure was 136 ± 16 mmHg, and did not differ between the groups. Although patients with CHD more often used statins (86% vs 73%, P=0.006), serum LDL was similar in the groups (2.0 ± 0.7 mmol/l), and serum HDL cholesterol was lower in those with CHD (1.0 ± 0.3 vs 1.2 ± 0.4 mmol/l, P<0.001) compared to no CHD.

Mean energy-adjusted dietary Mg intake was 305 ± 46 mg/day, and was lower in those with CHD (adjusted β=-0.14, P=0.003). Mean 24h urinary Mg excretion was 3.94 ± 2.05 mmol/24h and mean plasma Mg concentration was 0.77 ± 0.09 mmol/l, both did not statistically significantly differ among those with and without CHD (Table 1). Hypomagnesemia (plasma Mg <0.7 mmol/l) was present in 73 patients (17%), of which 11 patients (3%) had a plasma Mg <0.6 mmol/l. Dietary Mg intake was significantly correlated with 24h urinary Mg excretion (Pearson R=0.24, P<0.001), but not with plasma Mg (R=0.02, P=0.64). 24h urinary Mg excretion was significantly correlated with plasma Mg (R=0.13, P<0.008).

Systolic blood pressure was lowest in the highest gender-specific quartile of energy-adjusted magnesium intake (4th quartile 133 ± 13 vs 1st quartile 137 ± 17 mmHg; supplementary Table 2), and the number of antihypertensive drugs used was lowest in this quartile as well (4th quartile 2 [0-3] vs 2 [1-3] in other quartiles, P=0.008). Serum HbA1c and cholesterol levels were similar in all Mg intake quartiles. There was a trend towards higher urinary potassium excretion, dietary calcium, fiber, protein and carbohydrate intake, and lower dietary intake of fat in each higher quartile of magnesium intake.

*Association between dietary magnesium intake, 24h urinary magnesium excretion and plasma magnesium concentration and prevalence of coronary heart disease.*
The highest quartile of Mg intake was significantly associated with a lower prevalence ratio (PR) of CHD than the lowest quartile of Mg intake (0.40 (0.20, 0.77); Table 2). When adjusting for age and lifestyle parameters (BMI, smoking, alcohol and physical activity, the PR remained largely unchanged (0.42 (0.22, 0.82)). After adjustment for dietary intake of other micronutrients (total energy intake, sodium and potassium), the PR became (0.40 (0.20, 0.79)), and the P-trend was 0.01.

**Table 2.** Prevalence ratios (95% CI) for associations between dietary, urinary and plasma Magnesium and Coronary Heart Disease in type 2 diabetes from the DIALECT cohort (*n*= 450)

| | Quartile 1 | Quartile 2 | Quartile 3 | Quartile 4 | P-trend |
|---|---|---|---|---|---|
| Dietary Mg intake*, mg/day | 254 ± 25 | 291 ± 7 | 315 ± 8 | 361 ± 39 | |
| n cases/n total | 33/109 | 25/110 | 23/110 | 13/109 | |
| Model 1 | 1.00 | 0.71 (0.42, 1.22) | 0.64 (0.37, 1.10) | 0.40 (0.20, 0.77) | 0.005 |
| Model 2 | 1.00 | 0.72 (0.42, 1.23) | 0.69 (0.40, 1.21) | 0.42 (0.22, 0.82) | 0.01 |
| Model 3 | 1.00 | 0.71 (0.41, 1.23) | 0.72 (0.41, 1.27) | 0.40 (0.20, 0.79) | 0.01 |
| Urinary Mg excretion, mmol/24h | 1.81 ± 0.63 | 3.05 ± 0.32 | 4.32 ± 0.57 | 6.64 ± 1.75 | |
| n cases/n total | 24/101 | 24/100 | 19/101 | 15/100 | |
| Model 1 | 1.00 | 0.95 (0.54, 1.67) | 0.73 (0.39, 1.35) | 0.63 (0.33. 1.19) | 0.24 |
| Model 2 | 1.00 | 1.28 (0.71, 2.30) | 0.96 (0.51, 1.82) | 0.74 (0.39, 1.42) | 0.33 |
| Model 3 | 1.00 | 1.27 (0.70, 2.30) | 0.85 (0.44, 1.65) | 0.63 (0.32, 1.26) | 0.13 |
| Plasma Mg concentration, mmol/l | 0.67 ± 0.06 | 0.75 ± 0.02 | 0.80 ± 0.02 | 0.88 ± 0.04 | |
| n cases/n total | 29/113 | 22/106 | 27/111 | 16/102 | |
| Model 1 | 1.00 | 0.91 (0.52, 1.60) | 1.03 (0.60, 1.77) | 0.60 (0.31, 1.14) | 0.15 |
| Model 2 | 1.00 | 0.91 (0.51, 1.62) | 1.09 (0.63, 1.89) | 0.58 (0.30, 1.12) | 0.17 |
| Model 3 | 1.00 | 0.91 (0.51, 1.63) | 1.12 (0.65, 1.94) | 0.62 (0.32, 1.20) | 0.26 |

Model 1: Crude model; Model 2: Adjusted for age (years), BMI (kg/m$^2$), smoking (never, former or current), alcohol consumption (none, 1-13 units per week, ≥14 units per week), physical activity (not adherent to guideline, adherent to guideline); Model 3: Model 2 + Total energy intake (kcal), 24h urinary sodium excretion (mmol/24h) and 24h urinary potassium excretion (mmol/24h). * Dietary magnesium intake was adjusted for total energy intake using the residual method.

There was a similar trend towards a lower prevalence of CHD in the highest quartile of 24h urinary Mg excretion, which was not statistically significant (PR 0.63 (0.33. 1.19)). After adjustment for lifestyle and nutritional factors, the PR remained similar (0.63 (0.32, 1.26)). Also, the highest quartile of plasma Mg concentration had a non-significant trend towards a lower prevalence of CHD (unadjusted PR 0.60 (0.31, 1.14)); adjusted PR 0.62 (0.32, 1.20)). The PR ratios for dietary Mg intake, urinary Mg excretion and plasma Mg concentration did not change after further adjustment for the classical CHD risk factors systolic blood pressure and LDL cholesterol (data not shown).

*Analysis on source of magnesium intake and prevalence of CHD*

We performed an explorative analysis whether there was an association between Mg intake from specific dietary sources and CHD. The largest dietary contributors to total dietary Mg intake in T2D were (figure 1): cereals 22 [16-26] %, dairy 14 [10-20] %, coffee 9 [6-13] %, potatoes 7 [4-10] %, meat 6 [5-8] %, legumes and nuts 6 [4-11] %, fruit 5 [3-8] % and vegetables 3 [2-5] %. We found no statistically significant association between Mg intake from specific food groups and CHD (Table 3). However, there was a non-significant trend towards a lower prevalence of CHD for every 10mg increase of dietary Mg intake derived from vegetables (PR 0.75 (0.52, 1.08)).

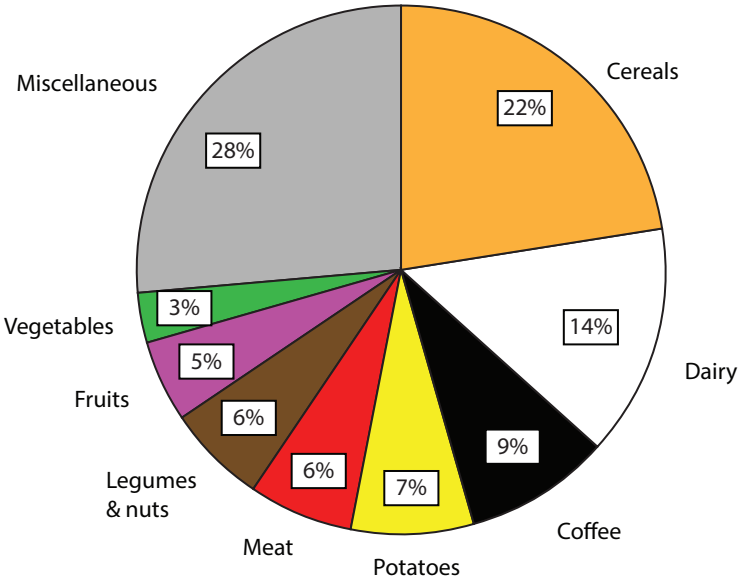


**Figure 1.** Sources of magnesium intake from different food product categories in patients with Type 2 Diabetes Mellitus.



**Table 3.** Prevalence ratios (95% CI) for associations between magnesium intake from different food sources in type 2 diabetes patients from the DIALECT cohort (*n*=450)

| | Model 1 | Model 2 | Model 3 |
|---|---|---|---|
| Source of magnesium intake | PR (95% CI) | PR (95% CI) | PR (95% CI) |
| Mg intake from cereals*, 10 mg/day | 1.02 (0.94, 1.10) | 1.02 (0.94, 1.10) | 0.95 (0.86, 1.05) |
| Mg intake from dairy*, 10 mg/day | 0.95 (0.87, 1.03) | 0.95 (0.87, 1.03) | 0.92 (0.84, 1.01) |
| Mg intake from coffee*, 10 mg/day | 0.95 (0.83, 1.06) | 0.95 (0.83, 1.08) | 0.96 (0.84, 1.10) |
| Mg intake from potatoes*, 10 mg/day | 1.03 (0.87, 1.22) | 1.02 (0.86, 1.21) | 0.97 (0.80, 1.16) |
| Mg intake from meat*, 10 mg/day | 0.91 (0.70, 1.20) | 0.91 (0.69, 1.19) | 0.80 (0.59, 1.09) |
| Mg intake from legumes & nuts*, 10 mg/day | 0.96 (0.89, 1.05) | 0.96 (0.88, 1.06) | 0.95 (0.86, 1.05) |
| Mg intake from fruit*, 10 mg/day | 1.00 (0.81, 1.23) | 0.98 (0.79, 1.20) | 0.96 (0.78, 1.19) |
| Mg intake from vegetables*, 10 mg/day | 0.71 (0.51, 1.01) | 0.71 (0.50, 1.01) | 0.75 (0.52, 1.08) |
| Mg intake from miscellaneous sources*, 10 mg/day | 0.95 (0.89, 1.02) | 0.95 (0.89, 1.03) | 0.90 (0.82, 0.99) |

Model 1: Crude model; Model 2: Adjusted for age (years), BMI (kg/m$^2$), smoking (never, former/current), alcohol consumption (none, 1-13 units per week, ≥14 units per week), physical activity (not adherent to guideline, adherent to guideline); Model 3: Model 2 + Total energy intake (kcal), magnesium intake from the other sources (cereals (mg/day), dairy (mg/day), coffee (mg/day), potatoes (mg/day), meat (mg/day), legumes & nuts (mg/day), fruit (mg/day), vegetables (mg/day) and other (mg/day)). * Magnesium intake from food sources was adjusted for total energy intake using the residual method. An increment of 10mg magnesium intake per day was used to calculate PR. PR, prevalence ratio; CI, confidence interval;

# Discussion

We found inverse associations for dietary Mg intake, 24h urinary Mg excretion and plasma Mg concentration, with the prevalence of CHD in patients with T2D. As far as we know, this is the first study in T2D patients which simultaneously reports on these three Mg parameters in relation to CHD. The inverse association between dietary Mg intake and the prevalence of CHD we found was strongest for Mg intake derived from vegetables, albeit not statistically significant.

The mean dietary Mg intake we report (305 ± 46 mg/day) was somewhat lower than the median Mg intake in the general Dutch population, which was around 350 mg/day[22], and was comparable to median Mg intake of population studies in the USA (308 mg/day) [4]. We found that cereals, dairy and coffee intake were the largest contributors to total Mg intake, 22%, 14% and 9% respectively. This was somewhat different from the UK population, where cereals (34%), meat (19%) and dairy (18%) intake were the most important contributors[23]. In contrast, in a US population the most important food groups were vegetables (13%), milk (8%) and meat (7%)[24]. It should be noted that different grouping of food products renders a head-to-head comparison between these percentages difficult. The 24h urinary Mg excretion we report (4.0 ± 2.1 mmol/24h) was in line with the general population the Netherlands (4.2 ± 1.7 mmol/24h for men and 3.5 ± 1.4 mmol/24h for women)[5]. Plasma Mg concentration (0.77 ± 0.09 mmol/l) was similar to an earlier report in Dutch diabetes patients (0.74 ± 0.10 mmol/l)[25]. The prevalence of hypomagnesemia we found (17%) was in the range of the reported prevalence of hypomagnesemia in T2D: 14 and 48%[10,11], and emphasizes that clinical vigilance for hypomagnesemia is warranted in T2D, because it is associated with increased insulin resistance and faster renal function decline[26].

We are the first to report an inverse association between dietary Mg intake and the prevalence of CHD in T2D. In contrast, a large meta-analysis in non-T2D patients demonstrated no association between dietary Mg intake and incident CHD[9]. However, low dietary Mg intake was associated with a higher risk of stroke, heart failure, new-onset diabetes, and all-cause mortality[9]. It is known that in T2D renal wasting of Mg occurs[27]. Additionally, Mg supplementation in T2D can improve insulin sensitivity and metabolic control[28]. Possibly, in T2D an adequate Mg intake is even more important than in non-T2D to maintain an adequate Mg status and prevent diabetes-related complications. These data fuel the hypothesis that magnesium intake is beneficial in T2D patients.

In addition, when investigating Mg intake from specific food sources, we found the strongest inverse association between Mg derived from vegetables and CHD, albeit not quite reaching statistical significance. To our knowledge, the association with vegetable derived Mg intake and CHD has not been described before. When studying Mg intake and Mg status it is important to consider bioavailability of ingested Mg for intestinal uptake, as this might vary considerably depending on the source of Mg intake[29]. Possibly, bioavailability from Mg in vegetables is larger than from other food sources, however this issue would have to be addressed an in-depth mechanistic study. Nevertheless, our data illustrate that when studying the association between micronutrients and outcomes, also intake of different food groups is important. As vegetable intake in our population was low[30], and vegetable intake only accounted for 3% of total Mg intake in this population, increasing vegetable intake is a good opportunity to not only increase Mg intake, but also improve overall diet quality. It should be noted that in our study, it is difficult to distinguish between protective effects from overall vegetable intake, or vegetable-derived Mg intake. Other vegetable-derived components, such as anti-oxidants, but also potassium, and vitamin K, might contribute to, or interact with Mg in the eventual association with CHD[31-34]. Maybe possible cohort effect from these micronutrients and Mg could amplify such protection. Because such an analysis is beyond the scope and beyond the available data of the current study, future studies are necessary to further investigate mechanisms behind vegetable intake and risk of coronary heart disease.



Additionally, we found that lower 24h urinary Mg excretion was associated with more prevalent CHD. In line, in the general population an inverse association between Mg excretion and CHD was reported[5]. Potential renal Mg wasting in T2D renders the interpretation of urinary Mg excretion difficult. High urinary Mg excretion could on the one hand reflect a high dietary Mg intake, but on the other hand could reflect hypermagnesuria found in T2D[10,11]. This could explain why, in our cohort, dietary Mg intake is more strongly associated with CHD than 24h urinary excretion.

In parallel, lower plasma Mg concentration was also associated with prevalent CHD. In T2D, the association between plasma Mg concentration and CHD was investigated previously, and conflicting results were reported[14,15]. In non-T2D, conflicting results on the association between plasma Mg have been reported as well, however a meta-analysis demonstrated an inverse association between plasma Mg and incident CHD[8]. As Mg is mainly an intracellular cation, and therefore plasma Mg only reflects 1% of bodily Mg stores, the validity of using plasma Mg as a marker for Mg status has been questioned; Mg deficiency has been reported in patients without overt hypomagnesemia[11,35].

Our paper was not designed to unravel mechanisms behind the inverse associations between Mg intake and Mg status and CHD. However, several mechanisms have been proposed that could underlie this association. First, animal studies have consistently shown that higher Mg status inhibits vascular calcification[36,37]. In human subjects, serum Mg and dietary Mg intake were inversely associated with degree of coronary calcification[4,6]. Second, low Mg status might be associated with cardiac arrhythmias[38]. Lastly, increased CHD risk might be mediated through the association between low Mg status/intake and increased traditional CHD risk factors, such as blood pressure[39] and insulin resistance[12].

Our paper is the first to simultaneously report the association between dietary Mg intake and factors of Mg status (24h urinary Mg excretion and plasma Mg) and CHD in patients with established T2D. The robustness of our findings is established through the fact that all three Mg parameters were inversely associated with CHD. The main limitation of our paper is the cross-sectional design, which only allows to study associations and not causality, and therefore there is a risk of reverse causality bias. Another limitation is that the FFQ we used in our study was not validated to estimate magnesium intake. However, because there was a moderate correlation between dietary Mg intake and urinary Mg excretion we deemed the results sufficiently valid.

Our study has several clinical implications. First, we show that Mg intake is of the utmost importance in T2D. Patients with T2D are at risk to develop hypomagnesemia, as Mg intake in our population was somewhat lower in comparison to the general population, and additionally, patients with T2D have increased renal Mg excretion[27]. We show that Mg intake and Mg status is reduced in those with CHD, possibly indicating that higher Mg intake is associated with a lower risk of CHD. The best opportunity to increase Mg intake is to increase intake of Mg-rich vegetables. As Mg intake in the highest quartile is approximately 100 mg/day higher than in the lowest quartile, in clinical practice this could correspond with increasing vegetable intake by for example 200g spinach, or 100g rucola lettuce and 2 avocados per day. Alternatively, previous research has shown that several dietary patterns might reduce CHD risk or improve cardiac function, such as the Mediterranean diet, DASH diet or a high protein, intermittent fasting low calorie diet[40-42]. We add to these findings by illustrating that Mg is an important components in such diets. For future studies, it would be of interest to investigate how Mg and other beneficial nutritional approaches could reinforce each other in the pursuit of reduction of CHD in diabetes patients. Additionally, future mechanistic studies should be done to investigate how vegetable-derived components, among which Mg, might reduce CHD risk.

## Conclusions

In a cohort of patients with established T2D, dietary magnesium intake, 24h urinary magnesium excretion and plasma magnesium concentration were inversely associated with the prevalence of coronary heart disease. Increasing dietary magnesium intake, especially through increasing vegetable intake, may reduce the risk of CHD in patients with established T2D.

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**Supplementary Table 1.** Overview of food items included in each food category

| | |
|---|---|
| 1 | **Cereals** |
| | Bread |
| | Rye bread |
| | Raisin bread |
| | Bread rolls |
| | Croissants |
| | Rusk |
| | Pasta |
| | Rice |
| 2 | **Dairy** |
| | Full milk |
| | Semi-skimmed milk |
| | Skimmed milk |
| | Buttermilk |
| | Other milk, such as horse and goat |
| | Normal milk in coffee |
| | Coffee milk, coffee cream |
| | Full fat milk beverages |
| | Semi-skimmed milk beverages |
| | Skimmed milk beverages |
| | Beverages with probiotics |
| | Unknown dairy drink |
| | Unknown type of milk |
| | Full (fruit) yogurt |
| | Semi-skimmed (fruit) yogurt |
| | Skimmed (fruit) yogurt |
| | Yogurt with probiotics |
| | Cholesterol-lowering yoghurt drink |
| | Full custard & pudding |
| | Low-fat custard & pudding |
| | Lean custard & pudding |
| | 20+/30+ cheese |
| | 40+ cheese |
| | Ordinary cheese (48+) |
| | Luxury fat cheese |
| | Low-fat cheese |
| | Unknown type of cheese |
| | Cheese used in hot meal |
| | Cheese as in between snack |
| | Full (fruit) cream cheese |
| | Semi-skimmed (fruit) cream cheese |
| | Skimmed (fruit) cream cheese |
| | Ice cream |
| | Whipped cream |
| | Cream used in hot meals |
| | Hot porridge |
| | Breakfast drink |
| | Butter |
| | Diet butter |
| 3 | **Coffee** |
| | Coffee |
| 4 | **Potatoes** |
| | Boiled/mashed potatoes without fat |
| | Fried/mashed potatoes with fat |
| | Oven-grilled french fries |
| | Deep fried french fries |
| | French fries prepared by someone else |
| 5 | **Meat** |
| | Chicken, turkey |
| | Beef, steak etc. |
| | Beef, blind finch, etc. |
| | Pork, steak etc. |
| | Pork, chop etc. |
| | Pork, smoked sausage etc. |
| | Lamb or mutton |
| | Other meats such as goat/horse |
| | Cooked liver |
| | Hepatic/renal products |
| | Sausages as in between snack |
| | Minced meat (all types) |
| | Liver products |
| | Ham etc. |
| | Lunch meats |
| | Bacon etc. |
| | Unknown type of meat |
| 6 | **Legumes and nuts** |
| | Legumes |
| | Split peas, beans etc. |
| | Split pea soup |
| | Nuts and seeds |
| | Peanuts (and coated peanuts) |
| | Nuts as in between snack |
| | Soy products |
| 7 | **Fruit** |
| | Citrus fruit |
| | Other fruit |
| | Fruit in can/jar |
| 8 | **Vegetables** |
| | Broccoli, cauliflower etc. |
| | Sal, spinach, endive etc. |
| | Mushrooms |
| | Onion, pepper, etc |
| | Raw vegetables |
| 9 | **Miscellaneous** |
| | Beverages |
| | Warm snacks |
| | Cold snacks |
| | Sauces |
| | Fish |
| | Peanut butter |
| | Jam |
| | Chocolate Paste |

**Supplementary Table 2.** Baseline characteristics of patients with type 2 diabetes mellitus by a breakup of dietary magnesium intake

| | | Quartile 1 |
|---|---|---|
| *Mean adjusted magnesium intake* | *305 ± 46* | *254 ± 25* |
| *Variable* | *n=450* | *109* |
| Age, years | 63 ± 9 | 63 ± 9 |
| Women, n (%) | 186 (43) | 46 (42) |
| Diabetes duration, years | 11 [7-18] | 11 [6-18] |
| Systolic blood pressure, mmHg | 136 ± 16 | 137 ± 17 |
| Diastolic blood pressure, mmHg | 74 ± 9 | 75 ± 10 |
| Heart rate, beats/min | 74 ± 13 | 74 ± 13 |
| Body surface area, $m^2$ | 2.10 ± 0.22 | 2.11 ± 0.24 |
| Urinary creatinine excretion, µmol/24h | 13.8 ± 4.8 | 14.1 ± 5.6 |
| *Complications* | | |
| Coronary heart disease, n (%) | 100 (22) | 33 (31) |
| Cerebrovascular disease, n (%) | 47 (11) | 14 (13) |
| Peripheral artery disease, n (%) | 44 (10) | 16 (15) |
| Retinopathy, n (%) | 106 (24) | 28 (26) |
| Neuropathy, n (%) | 157 (36) | 38 (35) |
| Diabetic nephropathy, n (%) | 183 (42) | 51 (47) |
| eGFR<60 | 101 (23) | 33 (30) |
| Microalbuminuria, n (%) | 131 (30) | 33 (31) |
| *Lifestyle* | | |
| Body mass index, $kg/m^2$ | 32.8 ± 6.2 | 33.2 ± 6.5 |
| Smoking, former or current, n (%) | 306 (70) | 76 (70) |
| Alcohol | | |
| No alcohol, n (%) | 148 (36) | 35 (34) |
| 0-13 units per week, n (%) | 206 (50) | 46 (45) |
| 14+ units per week, n (%) | 61 (15) | 22 (21) |
| Adherence guideline physical activity, n (%) | 249 (59) | 57 (54) |
| *Pharmaceutical treatment* | | |
| Insulin use, n (%) | 275 (63) | 65 (60) |
| Statin use, n (%) | 331 (76) | 83 (76) |
| β-blocker treatment, n (%) | 202 (46) | 60 (55) |
| RAAS inhibition, n (%) | 289 (66) | 74 (68) |
| Calcium antagonists, n (%) | 98 (22) | 27 (25) |
| Thiazide diuretics, n (%) | 136 (31) | 33 (30) |
| Loop diuretics, n (%) | 75 (17) | 22 (20) |
| Number of antihypertensives | 2 [1-3] | 2 [1-3] |

| Sex-specific quartiles of energy adjusted magnesium intake | | | |
|---|---|---|---|
| Quartile 2 | Quartile 3 | Quartile 4 | |
| *291 ± 7* | *315 ± 8* | *361 ± 39* | |
| *110* | *110* | *109* | |
| 64 ± 9 | 63 ± 8 | 63 ± 10 | 0.86 |
| 47 (43) | 47 (43) | 46 (42) | n.a. |
| 12 [7-19] | 11 [6-18] | 11 [7-16] | 0.68 |
| 139 ± 18 | 137 ± 16 | 133 ± 13 | 0.05 |
| 74 ± 9 | 76 ± 10 | 74 ± 9 | 0.50 |
| 73 ± 14 | 74 ± 12 | 75 ± 12 | 0.60 |
| 2.09 ± 0.21 | 2.08 ± 0.21 | 2.11 ± 0.21 | 0.67 |
| 13.8 ± 4.9 | 13.5 ± 4.1 | 13.8 ± 4.5 | 0.84 |
| | | | |
| 25 (23) | 23 (21) | 13 (12) | 0.007 |
| 11 (10) | 10 (9) | 12 (11) | 0.83 |
| 10 (9) | 7 (6) | 11 (10) | 0.23 |
| 38 (35) | 17 (16) | 23 (21) | 0.007 |
| 40 (36) | 36 (33) | 43 (39) | 0.77 |
| 49 (45) | 44 (40) | 39 (36) | 0.36 |
| 25 (23) | 24 (22) | 19 (17) | 0.16 |
| 39 (36) | 30 (27) | 29 (27) | 0.42 |
| | | | |
| 33.3 ± 6.3 | 32.5 ± 6.5 | 32.2 ± 5.5 | 0.49 |
| 80 (73) | 73 (66) | 77 (71) | 0.78 |
| | | | |
| 34 (32) | 39 (38) | 40 (40) | 0.40 |
| 58 (55) | 52 (50) | 50 (49) | |
| 14 (13) | 13 (13) | 12 (12) | |
| 64 (60) | 63 (58) | 65 (63) | 0.61 |
| | | | |
| 70 (64) | 65 (59) | 75 (69) | 0.42 |
| 80 (73) | 90 (82) | 78 (72) | 0.29 |
| 55 (50) | 46 (42) | 41 (38) | 0.04 |
| 78 (71) | 68 (62) | 69 (63) | 0.46 |
| 33 (30) | 21 (19) | 17 (16) | 0.06 |
| 42 (38) | 38 (35) | 23 (21) | 0.04 |
| 18 (16) | 16 (15) | 19 (17) | 0.73 |
| 2 [1-3] | 2 [1-3] | 2 [0-3] | 0.008 |

**Supplementary Table 2.** Continued

| | | Quartile 1 |
|---|---|---|
| *Mean adjusted magnesium intake* | *305 ± 46* | *254 ± 25* |
| *Magnesium values* | | |
| Urinary magnesium excretion, mmol/24h | 3.94 ± 2.05 | 3.94 ± 2.31 |
| Plasma magnesium concentration, mmol/l | 0.77 ± 0.09 | 0.77 ± 0.10 |
| Hypomagnesemia, n (%) | 72 (17) | 21 (20) |
| *Serum values* | | |
| Total cholesterol, mmol/l | 4.0 ± 0.9 | 4.0 ± 1.0 |
| HDL cholesterol, mmol/l | 1.1 ± 0.3 | 1.1 ± 0.4 |
| LDL cholesterol, mmol/l | 2.0 ± 0.7 | 2.1 ± 0.8 |
| HbA1c, mmol/mol | 57 ± 12 | 57 ± 12 |
| *Dietary intake* | | |
| Total energy intake, kcal/day | 1922 ± 629 | 2071 ± 665 |
| Urinary sodium excretion, mmol/24h | 185 ± 79 | 177 ± 82 |
| Urinary potassium excretion, mmol/24h | 77 ± 25 | 70 ± 24 |
| Calcium intake, mg/day | 969 ± 441 | 993 ± 456 |
| Fiber intake, g/day | 20.9 ± 6.6 | 19.4 ± 6.1 |
| Cholesterol, g/day | 194 ± 96 | 225 ± 126 |
| Total fat intake, g/day | 79 ± 39 | 89 ± 39 |
| Total protein intake, g/day | 79 ± 23 | 76 ± 24 |
| Total carbohydrate intake, g/day | 207 ± 69 | 217 ± 67 |

CHD coronary heart disease, eGFR estimated glomerular filtration rate (CKD-EPI), HDL high density lipoprotein, LDL low density lipoprotein, HbA1c glycated hemoglobin. Dietary magnesium intake was adjusted for total energy intake using the residual method.

| Sex-specific quartiles of energy adjusted magnesium intake | | | |
|---|---|---|---|
| Quartile 2 | Quartile 3 | Quartile 4 | |
| *291 ± 7* | *315 ± 8* | *361 ± 39* | |
| | | | |
| 3.57 ± 1.79 | 3.67 ± 1.81 | 4.61 ± 2.14 | 0.002 |
| 0.76 ± 0.09 | 0.77 ± 0.08 | 0.78 ± 0.08 | 0.20 |
| 25 (23) | 16 (16) | 10 (10) | 0.06 |
| | | | |
| 4.0 ± 0.9 | 3.9 ± 0.9 | 4.1 ± 1.0 | 0.82 |
| 1.1 ± 0.3 | 1.1 ± 0.3 | 1.2 ± 0.4 | 0.87 |
| 2.0 ± 0.7 | 2.0 ± 0.7 | 2.0 ± 0.8 | 0.86 |
| 56 ± 11 | 57 ± 11 | 59 ± 13 | 0.23 |
| | | | |
| 1797 ± 661 | 1850 ± 562 | 1973 ± 594 | 0.006 |
| 185 ± 79 | 174 ± 65 | 204 ± 88 | 0.03 |
| 75 ± 25 | 78 ± 26 | 86 ± 24 | <0.001 |
| 869 ± 441 | 982 ± 398 | 1150 ± 420 | <0.001 |
| 19.2 ± 6.6 | 21.1 ± 6.1 | 23.8 ± 6.6 | <0.001 |
| 193 ± 96 | 177 ± 75 | 183 ± 75 | 0.001 |
| 74 ± 32 | 73 ± 28 | 79 ± 33 | 0.001 |
| 73 ± 23 | 78 ± 21 | 87 ± 22 | <0.001 |
| 193 ± 76 | 206 ± 67 | 214 ± 63 | 0.05 |

# CHAPTER 6

## Physical activity in type 2 diabetes patients: the case for objective measurement in routine clinical care

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# Letter to the editor

To perform at least 150 min of moderate-to-vigorous physical activity (MVPA) per week is a major aim in type 2 diabetes treatment[1], but actual measurements are not routinely performed in clinical practice. We questioned whether subjective assessment of physical activity is accurate to guide lifestyle advices.

We compared the results of the Short Questionnaire to Assess Health–Enhancing Physical Activity (SQUASH)[2] and the Fitbit Flex accelerometer[3] in 50 patients with type 2 diabetes included in the DIAbetes and LifEstyle Cohort Twente (DIALECT) trial, which was approved by the local institutional review board METC-Twente (NL57219.044.16) [4]. Patients wore the Fitbit for 7 consecutive days and were instructed to maintain their usual activities. Raw Fitbit data (steps/min) were organized into ready variables by an algorithm written in MATLAB (MathWorks, Natick, MA). MVPA was defined as ≥95 steps/min[5]. Patients maintained a diary regarding activities not detected by the Fitbit (i.e., cycling, swimming, and fitness). Data are presented as median (interquartile range). The association between log-transformed measured minutes of MVPA was tested using linear regression analyses and the difference between MVPA with the Wilcoxon test for paired nonparametric data.

Median age was 70 (63–76) years, median diabetes duration was 16 (10–21) years, 74% of the patients were male, 82% used insulin, and 84% had microvascular and 42% had macrovascular complications. According to SQUASH, patients had 165 (0–645) min of MVPA/week, and 20 participants (40%) adhered to the American Diabetes Association (ADA) recommendation of ≥150 min of MVPA/week (figure 1). Fitbit data of >5 days were available in all patients. Median total steps per day were 4,277 (2,588–6,407), and 86% were able to reach ≥95 steps/min at some point during the measurement period. Based on the Fitbit data, patients had 23 (5–41) min of MVPA/week, and 1 (2%) participant adhered to the ADA guideline. When non-registered activity was added, the figures increased to 31 (5–72) min of MVPA/week and 7 (14%) patients fulfilling the guideline. There was an association between SQUASH-assessed minutes of MVPA/week and Fitbit-assessed minutes of MVPA/week ($\beta = 0.54$, $P<0.001$) (figure 1); however, the number of SQUASH assessed minutes of MVPA/week was significantly and substantially higher ($P<0.001$).

Subjective assessment grossly overestimated weekly MVPA compared with objective assessment. When self-reporting, roughly half of the patients seem to meet the MVPA recommendations, whereas objective measurements indicate this number is 14%, at best. A limitation is that the Fitbit Flex, while validated for measuring steps, does not register other activities. Currently, several types of activity trackers with additional functional-

ities have become available, and future research should evaluate which tracker is most appropriate for clinical use. Although it has not been tested as to whether overestimation of activity occurs in a wider array of patients with diabetes, the findings presented here clearly point out the importance of using objective measurements of activity. We propose to incorporate objective measurements of physical activity in the standard care for patients with type 2 diabetes. Such measurements can not only identify individuals at risk but also increase patients' awareness of physical inactivity and help to evaluate interventions.

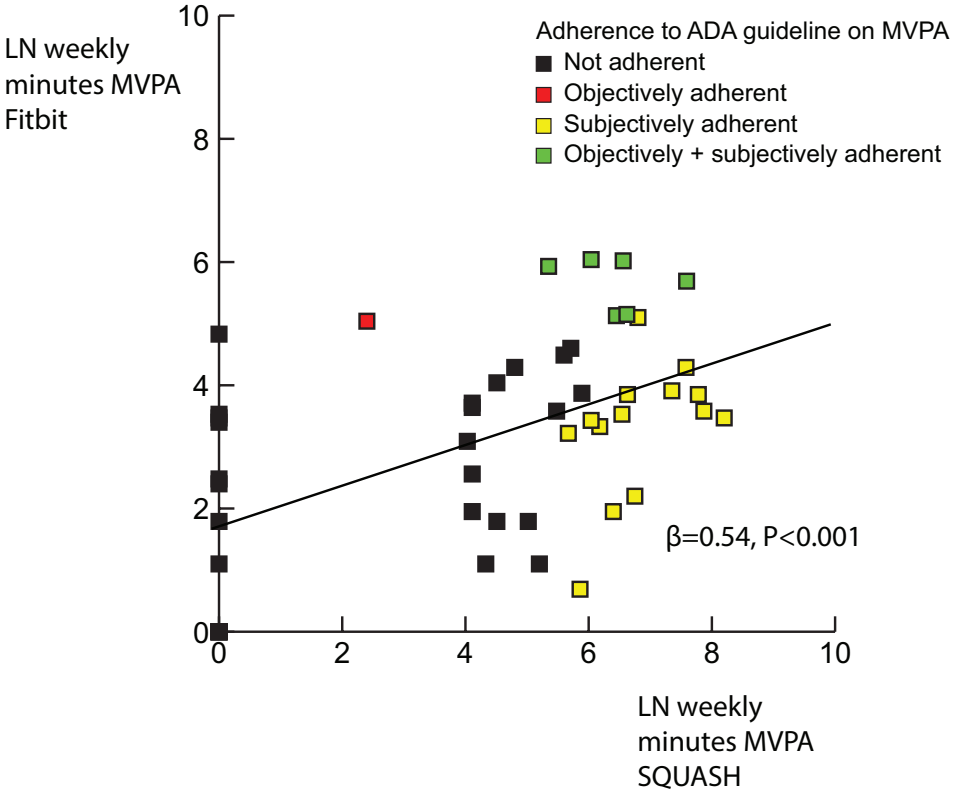


**Figure 1.** Minutes of MVPA measured with the SQUASH vs minutes MVPA measured with the Fitbit. There was a correlation between measured minutes of MVPA between the two methods, however in the majority of patients the absolute number of weekly minutes MVPA was substantially higher using the SQUASH results.

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# PART II

## Optimizing pharmacological treatment in cardiovascular risk management: neurohumoral activation in type 2 diabetes and chronic kidney disease

# CHAPTER 7

## MRA inhibition in CKD
more than salt and water

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# Abstract

Chronic kidney disease CKD) is one of the major causes of morbidity and mortality worldwide and it is closely related to cardiovascular disease. The most important causes of CKD in developed countries are diabetic nephropathy and renovascular disease/hypertension. Despite a proven favourable effect of RAAS blockade on short-term parameters (blood pressure, proteinuria) as well as long term outcome (slower decline of GFR), progression of chronic kidney disease to end stage renal disease still occurs in many patients. Mineralocorticoid receptor activation inhibition is currently being studied as a new therapeutic approach to CKD. Next to the classic anti-diuretic and potassium wasting effects, aldosterone has been shown to directly impact the heart, central nervous system, vasculature and kidneys, promoting inflammation, fibrosis and tissue remodelling, independently of its effect on sodium status and blood pressure. In this chapter we will describe the non-conventional renal and extrarenal effects of aldosterone, and review the effects of mineralocorticoid receptor antagonists in cardiovascular diseases and chronic kidney disease.

## Clinical case scenario

*A 54 year old man suffering from CKD (eGFR 58 ml/min•1.73m²) was treated with the maximum dose of an ACE inhibitor. He had no primary glomerular disorder necessitating specific immunosuppressive treatment. However, proteinuria (4.8 g/day, target <1.0 g/day) and ambulatory blood pressure (155/96 mmHg) were insufficiently controlled. He was sent to a dietician and instructed to adhere to a moderate sodium restriction. He successfully changed his habits, reflected by a decrease in urinary sodium excretion from 245 mmol/ day to 150 mmol/day, and a concomitant improvement of proteinuria (2.6 g/day) and blood pressure (145/88 mmHg), both typical for the amount of sodium restriction. As proteinuria and blood pressure were still above target, the decision was then made to add the MRA inhibitor spironolactone 25 mg once daily. The patient was extensively instructed to stop the drug in any circumstance of excessive water/sodium loss. The proteinuria (1.1 mmol/day) and blood pressure (132/81 mmHg) further decreased. There was an acceptable decrease in eGFR from 58 to 51 ml/min•1.73m² (reflecting the reversible effect of treatment on glomerular pressure) and renal function remained stable in three subsequent years. Based on the 24 h urine collections, he was reinforced to keep his sodium restriction on a few occasions. In the next summer, he suffered from a severe gastroenteritis after having a barbecue. He immediately stopped spironolactone and promptly contacted the outpatient clinic for blood collection. The potassium concentration remained within acceptable limits. After recovery of his illness, spironolactone was started again.*

# Introduction

Chronic kidney disease (CKD) is characterized by prolonged (≥3 months) structural and functional abnormalities of the kidneys. CKD is defined by a decreased renal function, that is glomerular filtration rate (GFR) <60 ml/min•1.73m$^2$, and/or urinary loss of protein. The latter, proteinuria, may also be measured as albuminuria.

CKD is one of the major causes of morbidity and mortality worldwide and it is closely related to cardiovascular disease. CKD prevalence has been steadily increasing over the last decades, leading to a large global disease burden. The most important causes of CKD in developed countries are diabetic nephropathy and renovascular disease/hypertension. Deterioration of renal function into end stage renal disease (ESRD) leads to a requirement for renal replacement therapy, i.e. dialysis or kidney transplantation. Unfortunately, the possibility for transplantation is limited by donor shortage, and dialysis is associated with a poor quality of life, partly caused by an extremely increased risk of cardiovascular complications. Prevention of renal function loss in the earlier stages of CKD is therefore of utmost importance.

The so-called renoprotective treatment aims to halt kidney damage and thus progressive renal function loss with blood pressure and proteinuria as important intermediate outcome measures. Therapy of choice is blockade of the renin-angiotensin-aldosterone system (RAAS), with either angiotensin converting enzyme inhibitors (ACEi), or angiotensin II receptor blockers (ARB). Despite a proven favourable effect of RAAS blockade on short-term parameters (blood pressure, proteinuria) as well as long term outcome (slower decline of GFR), progression of CKD to ESRD still occurs in many patients. In the search for additional treatment options, combination therapy by ACEi and ARB, or combination of either ACEi or ARB with direct renin-inhibitor, unfortunately do not improve long term renal outcome, and may even worsen it.

Mineralocorticoid receptor activation (MRA) inhibition is currently being studied as a new therapeutic approach to CKD. Although this therapy is not new, there is new attention, fuelled by striking new insights that have been obtained in the understanding of aldosterone and its (patho)physiological effects. Next to the classic anti-diuretic and potassium wasting effects, aldosterone has been shown to directly impact the heart, central nervous system, vasculature and kidneys, promoting inflammation, fibrosis and tissue remodelling, independently of its effect on sodium status and blood pressure. Furthermore, it has been shown that during prolonged RAAS blockade treatment, aldosterone levels return to normal only in 10-50% of cases, suggesting a window of opportunity for intervention. Here we will review the role of MRA inhibition as an added treatment option in the management of chronic kidney disease.

# Aldosterone

*Classic effects and regulation of aldosterone release.*
Aldosterone, primarily synthesized in the zona glomerulosa of the adrenal cortex, is a steroid hormone with mineral corticoid activity. In addition, aldosterone is also locally synthesized in the blood vessels, brain, heart and adipocytes. Adrenal release of aldosterone is stimulated by an increase in angiotensin II, by hyperkalaemia and by the adrenocorticotropic hormone (ACTH). Circulating aldosterone levels are higher in men than in women, but the clinical significance of this difference is so far unknown.

The classic role of aldosterone is homeostatic volume control by promoting sodium and fluid reabsorption in the kidneys, thus providing a main contribution to the body's defence against sodium/ volume depletion. Furthermore in hyperkalaemia aldosterone promotes potassium excretion in the kidneys and colon. These classical effects are achieved by binding to the mineralocorticoid receptor (MR), which is located in the cortical collecting ducts in the distal nephron. The MR is a cytosolic receptor which migrates to the cell nucleus when ligand-activated. Here it attaches to the hormone regulatory part of target genes, enhancing transcription and translation of these genes, and thus stimulating the synthesis of aldosterone-induced proteins. The subsequent molecular pathway leads to an increased expression of the $Na^+/K^+$ pump in the apical epithelial membrane of the cell, increasing sodium reabsorption and potassium wasting. Furthermore, it increases expression of the basolateral $Na^+/K^+$-ATPase, stimulating sodium extrusion out of the cell and potassium entry into the cell. Lastly it increases the expression of apical renal outer medullary potassium channels which are involved in passive excretion of potassium. The MR has equal affinity to both mineral corticosteroids and glucocorticosteroids. While glucocorticoid plasma levels greatly exceed aldosterone plasma levels, the enzyme 11β hydroxysteroid dehydrogenase-2 metabolizes intracellular glucocorticosteroid levels from 100- to 10-fold that of aldosterone, rendering aldosterone the major activator of the MR.

*Non-classic effects of aldosterone.*
In the last decades new renal and extra-renal effects of aldosterone have been found, which can be reversed by MRA inhibition (figure 1). It has become known that aldosterone not only leads to genomic effects through activation of the MR, but can also lead to rapid non-genomic effects which do not require the transcriptional pathway described earlier[1,2]. These non-genomic effects consist primarily of pro-fibrotic and pro-inflammatory changes and are mediated through (an interplay of) the cytosolic MR and aldosterone receptors in the cell membrane (figure 2). Aldosterone exerts these effects on not only renal targets, such as podocytes, mesangial cells and renal vasculature, but also on extra-renal targets where the MR has been found, primarily cardiomyocytes, endothelial cells, vascular smooth muscle cells, adipocytes, and macrophages[3].

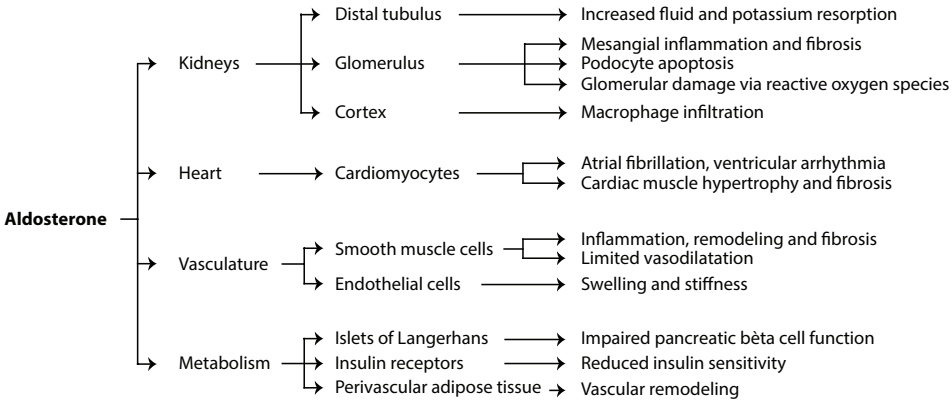


**Figure 1.** Aldosterone exerts effects in the kidney, the heart, the vasculature and on the metabolism. It binds to the mineralocorticoid receptor in various target cells to induce different effect mechanisms.

Aldosterone is pathophysiologically involved in kidney damage through multiple mechanisms in addition to the effects of elevated systemic blood pressure[4,5] (figure 2). Firstly, glomerular damage is induced by the increased production of reactive oxygen species (ROS) by mitochondria[6,7]. Furthermore, aldosterone increases expression of the pro-inflammatory serine/threonine-protein kinase and activates NFkB, which ultimately lead to mesangial inflammation, fibrosis, and glomerular injury[8]. Also macrophage infiltration into the renal cortex is stimulated by aldosterone, which promotes inflammation[9]. Lastly increased local renal aldosterone production has been shown to induce apoptosis in podocytes, through mechanisms which are not completely elucidated[10]. These local effects were larger in rats with diabetes mellitus, which was associated with increased MR and aldosterone levels. All the mechanisms listed above may contribute to renal damage and consequent deterioration of kidney function. Indeed, an increased proteinuria has been found in patients with excess aldosteronaemia, independent of blood pressure[4,5]. As proteinuria is a bad prognostic sign for CKD, this adds to the rationale for aldosterone inhibition as a new promising approach in halting disease progression.

The heart was the first extra-renal organ where mineralocorticoid receptors were found. Here aldosterone has been shown to promote electrophysiological remodeling, leading to atrial fibrillation and ventricular arrhythmias[3]. Remodeling takes place through modulation of T-type, potassium L-type and ryanodine receptor calcium channel activity[11-14]. Furthermore through pro-inflammatory processes cardiac hypertrophy and cardiac fibrosis are propagated, resulting in a reduced cardiac function. The blood vessels are a target for aldosterone through MRs in the vascular smooth muscle cells and endothelial cells.

Here too hyperaldosteronism can lead to tissue inflammation, remodeling and fibrosis. MRA leads to endothelial swelling and stiffness by promoting the insertion of the epithelial sodium channel (ENaC) into the cell membrane[15]. Due to a simultaneous decreased ability to form nitric oxide, vasodilatation is limited. There is evidence that this is a main pathophysiological event leading to hypertension caused by hyperaldosteronism, rather than excess fluid retention.

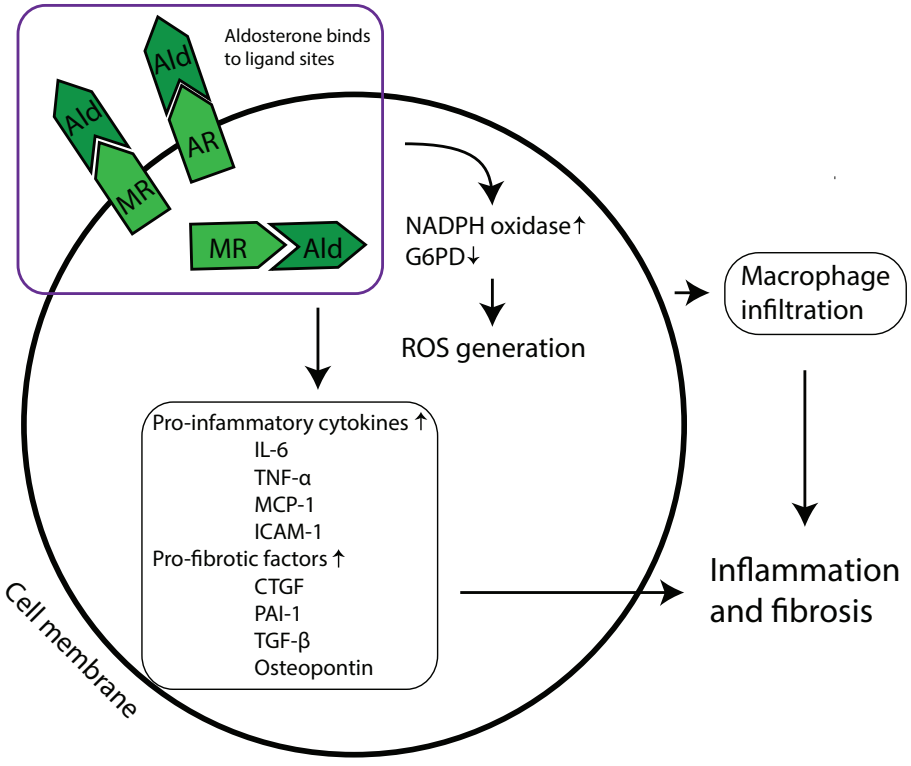


**Figure 2.** Stimulation of cytosolic and membrane MRs by aldosterone in combination with stimulation of aldosterone receptors in the cell membrane lead to pro-inflammatory and pro-fibrotic effects[2]. In the mitochondria NADPH oxidase activity is increased, while G6DP activity is reduced, stimulating ROS generation[3]. Furthermore aldosterone upregulates pro-inflammatory and pro-fibrotic cytokines, and induces macrophage infiltration[5]. Ultimately these changes lead to tissue inflammation and fibrosis. MR, mineralocorticoid receptor; Ald, aldosterone; AR, aldosterone receptor; G6PD, glucose-6-phosphate dehydrogenase; ROS, reactive oxygen species; IL-6, interleukin; TNF-α, tumor necrosis factor; MCP-1, monocyte chemoattractant protein; ICAM-1, intercellular adhesion molecule; CTGF, connective tissue growth factor; PAI-1, plasminogen activator inhibitor; TGF-b, transforming growth factor.

Excess aldosterone levels are associated with the cardiometabolic syndrome, which is characterized by insulin resistance, central obesity, dyslipidemia and hypertension[3,16]. Aldosterone impairs pancreatic beta cell function by promoting inflammation and oxidative stress in the islets of Langerhans[17,18]. Also aldosterone degrades insulin receptor substrate proteins, reducing insulin sensitivity and glucose-uptake[19]. Furthermore, aldosterone promotes the release of inflammatory cytokines from adipose tissues resulting in systemic inflammation and impaired glucose tolerance[18]. The MR is found in adipocytes and perivascular adipose tissue (PVAT). Adipocytes and PVAT can produce local levels of aldosterone which exert paracrine and autocrine effects, influencing not only adipose tissues but also the vasculature and thus promoting vascular remodelling. In line, aldosterone blockade has been shown to be particularly effective to reduce blood pressure in obesity–related hypertension[20].

*The interaction between salt status and aldosterone effects.*

The activity of the RAAS depends on what is denoted to as the "salt status", the RAAS being suppressed in state of sodium/fluid excess and being activated in a state of sodium depletion. Interestingly, an interaction between salt status and the pro-fibrotic, pro-inflammatory effects of aldosterone has been described, which is independent of blood pressure[21]. While in rats on a high or normal salt diet, high levels of aldosterone are associated with development of substantial end organ damage, high levels of aldosterone do not have such target organ effects during a low salt diet. Furthermore, the hypertrophic effects observed in high-salt, high-aldosterone rats can be completely reversed by the addition of MRA inhibitors, underlining the causal role of the mineralocorticoid receptor in the pathophysiological process. Salt excess has a role in sensitizing cardiovascular tissue for damage caused by an excess in aldosterone through mechanisms currently unknown. Interestingly new research shows that in CKD patients urinary salt excess is a significant predictor of urinary excretion of the mineralcorticoid metabolites tetrahydroaldosterone and tetrahydrocorticosterone, suggesting an alternative regulatory mechanism for aldosterone[22]. Whereas this novel insight may provide a missing link between CKD, high salt-status, and increased target organ damage, further research regarding this topic is warranted.

# MRA inhibition

*MRA inhibition in heart failure.*

After publication of the results from the large randomized controlled trials RALES and EPHESUS, MRA inhibition has been adopted as part of the standard treatment of chronic heart failure[23,24]. These studies showed that MRA inhibition with spironolactone or eplerenone reduces morbidity and mortality in patients with severe chronic heart failure (NYHA functional class III and IV; Table 1). The EMPHASIS-HF study showed that eplerenone was also effective in patients with mild symptoms of heart failure (NYHA functional class II)[25]. This is supported by a meta-review published in 2009 which showed a reduction in all-cause mortality and an increase in ejection fraction in patients with left ventricular failure on spironolactone therapy[26]. However, in a recent observational study spironolactone was shown not to have a significant effect on risk of hospitalization and death, suggesting that the benefits of MRA inhibition that has been found in randomized trials may not be true in clinical practice[27]. However, in this study outcome was not adjusted for baseline disease severity and spironolactone use may have been more often applied in patients with a worse clinical condition. Currently the ACCF/AHA recommends MRA inhibition therapy in patients with NYHA class II to IV heart failure and a left ventricular ejection fraction of ≤35%, unless contraindicated[28].

*The role of MRA inhibition in CKD treatment.*

Current treatment of CKD aims to prevent progressive renal function loss and its associated cardiovascular complications. Treatment of hypertension and proteinuria is the cornerstone of renoprotection. Inhibition of the RAAS by either ACEi or ARB has been proven to be an effective treatment option in CKD, reducing proteinuria as well as the rate of renal function loss. However, additional treatment options are necessary as progression of kidney disease still occurs in many patients. Combined blockade of the RAAS at different levels has been tested in several combinations, mostly ACEi combined with ARB[29]. Whereas dual blockade, with either ACEi plus ARB, or ACEI plus a renin-inhibitor is associated with a better efficacy on short term (i.e. proteinuria), hard outcome studies show that dual RAAS blockade does not confer better reno- and cardio-protection, but to the contrary, is associated with worse outcome[30].

Another strategy to improve the efficacy of RAAS-blockade, is in manipulating the salt status: correction of the volume overload in patients on monotherapy RAAS-blockade, by either diuretics, a low sodium diet or their combination, considerably potentiates the efficacy of monotherapy RAAS-blockade[31,32] (and Kwakernaak, Lancet 2014, in press). Post-hoc analyses suggest that moderate dietary sodium restriction is also associated with better long-term outcome of RAAS-blockade[33], but prospective studies, so far,

are unfortunately lacking. Observational data have shown a worse long term outcome in subjects that consume very low (<5 g/d) amounts of salt[34]. Whereas this might be related to underlying conditions associated with poor intake and malnutrition, it has also been pointed out that reactive hyperreninemic hyperaldosteronism might exert an adverse effect in such subjects[35] (figure 3). Of note, despite interference with the renin-angiotensin axis, a low sodium diet during ACEi or ARB is associated with secondary hyperreninemic aldosteronism that is particularly marked during the combination of diuretics and low sodium, demonstrating that the feedback loop between volume status and aldosterone is not disrupted by the current modes of inhibition of the RAAS by ACEi or ARB.

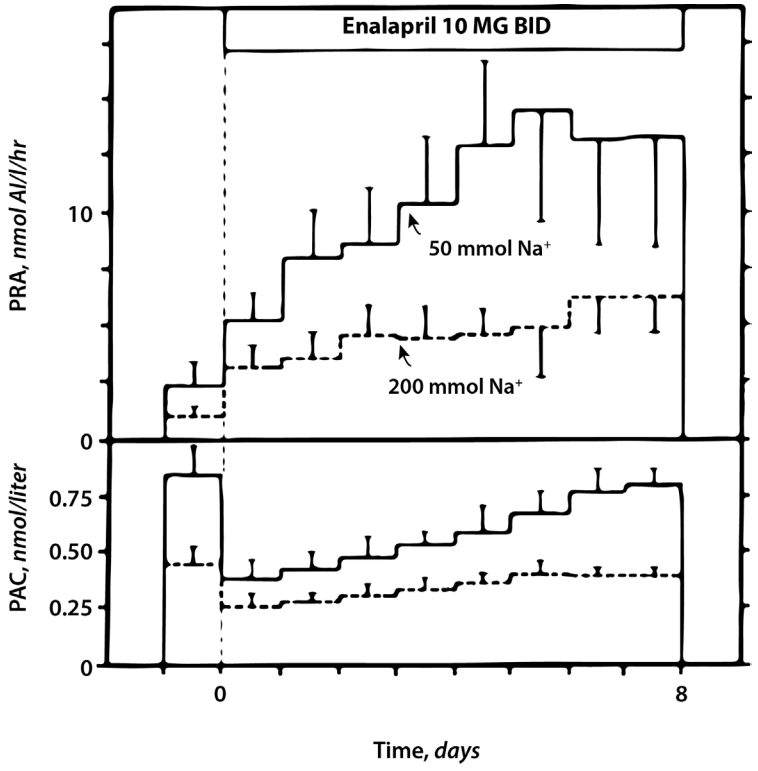


**Figure 3.** Effects of enalapril on PRA and PAC (mean ± SEM) on liberal (broken lines) and low (continuous lines) sodium diet. PRA: plasma renin activity; PAC: plasma aldosterone concentration. (Reprinted by permission from Macmillan Publishers Ltd: Navis *et al.* [37])

It has long been known that while under RAAS inhibition treatment with ACEi or ARB, aldosterone levels initially decrease, but can thereafter gradually increase to pretreatment, or even exceed pretreatment levels, a phenomenon that has been called "aldosterone

breakthrough" or "aldosterone escape"[36] (figure 3). Whereas this so-called escape is usually associated with long term treatment, detailed assessment of the early effects of RAAS-blockade on aldosterone levels demonstrate that, actually, aldosterone displays an early partial return towards its baseline values even during the first week of treatment, possibly in response to the negative sodium balance induced by the RAAS-blockade, again demonstrating the preservation of aldosterone's role in volume homeostasis, despite ACE-inhibition [37].

Keeping in mind the deleterious effects of aldosterone on the kidneys, MRA inhibition is a promising new therapeutic approach in CKD. Studies in experimental animals, in proteinuric models and in CKD patients support a renoprotective effect of MRA inhibition. As monotherapy RAAS-blockade is therapy of choice for CKD, in particular the added effects of MRA to ACEi or ARB are of clinical interest. These could be due to interference with classic effects of aldosterone (i.e a diuretic effect of MRA) as well as due to interference with the non-classic effects of aldosterone. A Cochrane review dating from 2009 has shown that MRA antagonism in addition to an ACEi or ARB, can in small doses significantly reduce proteinuria while only slightly influencing renal function or blood pressure, with the adverse effect of an increased incidence of hyperkalemia (Table 1)[38]. Long term clinical endpoints such as cardiovascular events or renal function were not available in the included studies. A recent systematic review concerning the effect of MRA inhibition in addition to RAAS inhibition on diabetic nephropathy confirmed these effects on proteinuria, blood pressure, glomerular filtration rate and potassium levels, however data on long term cardiovascular and renal outcome were still not available[39]. In non-diabetic patients with CKD, long term effects of MRA inhibition in addition to RAAS inhibition are also unavailable, while short term effects are comparable to that in patients with diabetic nephropathy[40]. In patients with mild CKD, treatment with MRA inhibitors was shown to have a beneficial effect on left ventricular mass and arterial stiffness[41]. In summary, the short-term effects of spironolactone and eplerenone are beneficial for CKD patients and these agents are also safe, provided that potassium levels are being monitored. However, there is a lack of data concerning the long term clinical outcome of dual treatment with an ACE-inhibitor or ARB and a MRA inhibitor, as well as lack of data on the mechanism of the added effect of MRA to ACEi or ARB. Therefore more research is needed to determine the definite role of MRA inhibition in CKD treatment.

Table 1. Long term effects of MRA inhibition in several types of disease

| *Author* | *Study type* | *Studied population* | *No of patients* | *Baseline medication* |
|---|---|---|---|---|
| HEART FAILURE | | | | |
| Pitt *et al.* 1999[23] | Randomized controlled trial | NYHA III and IV heart failure | 1663 | ACEi, loop diuretic |
| Pitt *et al.* 2003[24] | Randomized, double-blind, placebo-controlled trial | Acute myocardial infarction complicated by LV dysfunction and heart failure | 6632 | Optimal treatment |
| Zannad *et al.* 2011[25] | Randomized, double-blind placebo-controlled, parallel-group trial | NYHA II heart failure | 2737 | ACEi, ARB, or both, and a beta-blocker |
| Lee *et al.* 2013[27] | Prospective cohort study | Newly diagnosed heart failure, LVEF <40% | 2538 | ACEi, ARB, beta-blocker, loop diuretic and/or calcium channel blocker |
| CHRONIC KIDNEY DISEASE | | | | |
| Navaneethan *et al.*2009[38] | Cochrane systematic review | CKD patients | 845 | ACEi or ARB |
| Mavrakanas *et al.* 2013[39] | Systematic review | Diabetic nephropathy | 404 | ACEi or ARB |
| Boesby *et al.* 2011[40] | Open randomized cross-over trial | Non-diabetic chronic kidney disease | 40 | ACEi or ARB |

| | *MRA* | *Follow up* | *Endpoints* | *Results* | *Hyperkalemia* |
|---|---|---|---|---|---|
| | Spironolactone 25mg daily | 24 months | Death from any cause | RR of death, 0.70; 95% CI 0.60 to 0.82, P<0.001; RR of hospitalization, 0.65; 95% CI 0.54 to 0.77, P<0.001 | Minimal incidence of serious hyperkalemia |
| | Eplerenone 25-50mg daily | 16 months | Death from any cause | RR of death, 0.85; 95% CI 0.75 to 0.96, P=0.008 | Serious hyperkalemia, 5.5% vs 3.9% in the placebo group (P=0.002) |
| | Eplerenone up to 50 mg daily | 21 months | Death from CV causes or a first hospitalization for HF | HR of deatb from CV cause or first hospilization for HF, 0.63; 95% CI 0.54 to 0.74, P<0.001 | Serious hyperkalemia, 11.8% vs 7.2% in the placebo group P<0.001 |
| | Spironolactone | 36 months | Death from any cause, hospitalization | Adjusted HR of death, 0.93, 95% CI 0.60 to 1.44; adjusted HR of hospitalization 0.91, 95% CI 0.77 to 1.08 | Severe hyperkalemia 4.8 per 100 person-years vs 1.6 per 100 person-years with nonuse, p <0.001 |
| | Spironolactone | 2-12 months | Proteinuria, GFR, BP | 24 hour proteinuria, 7 studies, 372 patients; MD -0.80 g, 95% CI -1.23 to -0.38. eGFR, 5 studies, 306 patients: MD -0.70 ml/min•1.73m$^2$, 95% CI -4.73 to 3.34. Systolic BP, 7 RCTs, 372 patients: MD -3.40 mm Hg, 95% CI -5.13 to -1.68. Diastolic BP, 6 studies, 336 patients: MD -1.79 mm Hg, 95% CI -2.99 to -0.59 | Hyperkalemia, 8 studies, 436 patients: RR 3.06, 95% CI 1.26 to 7.41 |
| | Spironolactone 25mg daily or eplerenone 50-100mg daily | 2-12 months | Proteinuria, GFR, BP | Reduction of albuminuria by 23-67%. GFR slightly decreased. Significant drop in BP | Increased incidence hyperkalemia |
| | Eplerenone 25-50mg daily | 2 months | Albuminuria, creatinine clearance, BP | Urinary albumin excretion was 22% lower, CI: 14,28, P,0.001; creatinine clearance 5% lower, CI: 2,8, P=0.005; mean systolic BP 4 mmHg lower, CI: 2,6, P=0.002; diastolic BP 2 mmHg lower, CI: 0,4, P=0.02 | Potassium 0.1 mEq/L higher, CI: 0.1,0.2, P<0.001 |

*Aldosterone breakthrough and sodium status intervention in CKD.*
The importance of aldosterone breakthrough during RAAS blockade treatment on clinical endpoints is currently unknown. While a low salt diet is associated with higher aldosterone levels, the deleterious effects of aldosterone on end organs are generally absent during low salt-status. In contrast, in patients with a high salt intake, however, aldosterone breakthrough may be associated with an increase in target organ damage. Better elucidation of the interrelationships between salt status and the renoprotective effects of aldosterone blockade in CKD is therefore needed. This could also contribute to a better understanding of the mechanisms of the added effects of MRA to ACE-inihibition or ARB. In a best-case scenario MRA inhibition could be an add-on therapy that allows to combine ACE-inhibition or ARB with low sodium diet potentiating its renoprotective effects, without the possible adverse effects of a reactive rise in aldosterone. Several studies on the renoprotective effects of MRA inhibition are currently ongoing, including long term intervention for prevention of diabetic nephropathy[42] and a short-term intervention analysing the role of sodium status, and comparison with conventional diuretic during RAAS-blockade. These results may provide insight in the complex interplay between aldosterone breakthrough, salt status and MRA inhibition and thus delineate the optimal use of MRA for renoprotection

*Effects on vascular fibrosis and hypertension.*
Spironolactone and eplerenone are anti-hypertensive agents used in patients with resistant essential hypertension. The rationale behind the use of MRA inhibitors for treating hypertension is derived from the classical genomic effects of aldosterone. By blocking the MR, slow onset volume and sodium loss occurs, and thusly blood pressure is lowered. However, in the absence of activation of the MR in vascular tissues, also arterial stiffness induced by aldosterone can be prevented. In 2008 a study has shown that treatment with eplerenone can reduce the media collagen/elastin ratio, reducing vascular stiffness[43]. Also, in low-renin hypertensive patients eplerenone was shown to be more effective than the ARB losartan in lowering blood pressure[44]. Possibly, in a low-renin state the MRA inhibitor is more efficient in reducing the non-classical effects of aldosterone than ARB. However, in diabetic patients spironolactone was shown not to influence endothelial function, while effectively reducing blood pressure[45]. While MRA inhibitors are effective in reducing blood pressure, more research is needed to clear up the physiological mechanisms.

*MRA inhibition and PTH.*

Primary hyperparathyroidism is a known risk factor for developing cardiovascular disease. Recent findings have suggested a bilateral interplay between aldosterone and parathyroid hormone levels. Aldosterone secretion in the zona glomerulosa of the adrenal gland is regulated by Ca2+ channels[46]. There is evidence PTH levels can up-regulate aldosterone by increasing plasma calcium[47]. On the other hand, relative hyperaldosteronism stimulates urinary and faecal Ca2+ excretion in the presence of excess dietary salt intake, and thusly stimulates PTH secretion[48]. Recently the MR was also identified in parathyroid cells[49]. It is unknown, however, whether activation of these MRs also lead to increased PTH excretion. The combination of relative hyperaldosteronism and elevated PTH levels can increase target organ damage, as well as have deleterious skeletal effects. Research has shown that patients with congestive heart failure treated with spironolactone have a reduced fracture risk compared with patients without MRA inhibition treatment[50]. Recently the relationship between the RAAS and the PTH level in humans, without primary hyperaldosteronism has been studied, and it was shown that spironolactone treatment can slightly but statistically significantly reduce plasma PTH levels[51]. However in this study aldosterone infusion did not directly increase PTH levels, suggesting a more long term gradual effect of aldosterone on PTH levels. As secondary hyperparathyroidism often occurs in both CKD and congestive heart failure patients, the interplay between PTH and aldosterone may be relevant[52]. Future research will have to determine the role of PTH on end organ damage, and the effect of MRA inhibitors on PTH and calcium metabolism.

*Adverse effects of MRA inhibition.*

The most important adverse events associated with MRA inhibition therapy are hyperkalaemia and hemodynamically mediated renal function loss. Most trials concerning MRA inhibitor usage show an increase in hyperkalaemic events in patients treated with mineralocorticoid receptor antagonists, however, this was not associated with an increased risk of hospitalization or death in patients with heart failure or CKD[27,53]. Furthermore, significant or irreversible renal function loss was not seen. Still, long-term studies on the effect of MRA inhibition in CKD patients on renal function and cardiovascular events are currently lacking.

## Conclusion

New insights in the non-genomic effects of aldosterone on multiple target organs render MRA inhibition a promising approach in CKD treatment. The role of aldosterone breakthrough during RAAS blockade therapy is under investigation. While salt restriction is associated with higher aldosterone levels during ACEi or ARB, low salt intake could prevent the non-classical pro-fibrotic pro-inflammatory effects of aldosterone. Future studies will have to answer the question whether there is a role for MRA inhibition in CKD treatment in addition to current evidence-based renoprotective treatment (RAAS blockade with ACEi or ARB, salt restriction). Furthermore, upcoming data on the efficacy of MRA inhibition as compared to conventional diuretics, and the optimal combination with dietary salt targeting in CKD will allow the understanding needed to use MRA inhibition for optimal therapeutic benefit in CKD patients.

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# CHAPTER 8

## Gender Differences in Renin Angiotensin Aldosterone System Affect Extra Cellular Volume in Healthy Subjects

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# Abstract

**Objective** Several studies reported gender differences in aldosterone. It is unknown whether these differences are associated with differences in volume regulation. Therefore, we studied both aldosterone and extracellular volume in men and women on different sodium intakes.

**Methods** In healthy normotensive men ($n$=18) and premenopausal women ($n$=18) we investigated plasma aldosterone, blood pressure, and extracellular volume (125I-iothalamate), during both a low (target intake 50 mmol $Na^+$/day) and high sodium intake (target intake 200 mmol $Na^+$/day) in a cross-over set-up. Furthermore, we studied the adrenal response to angiotensin II infusion (0.3, 1.0 and 3.0 ng/kg/min for 1 h) on both sodium intakes.

**Results** Men had a significantly higher plasma aldosterone, extracellular volume and systolic blood pressure than women during a high sodium intake ($p<0.05$). During a low sodium intake, extracellular volume and blood pressure were higher in men as well ($p<0.05$), whereas the difference in plasma aldosterone was no longer significant ($P=0.252$). The adrenal response to exogenous angiotensin II was significantly lower in men than in women on both sodium intakes.

**Conclusions** Constitutive gender differences in the regulation of aldosterone, characterized by a higher aldosterone and a lower adrenal response to exogenous angiotensin II infusion in men, are associated with a higher extracellular volume and blood pressure in men. These findings suggest that gender differences in the regulation of aldosterone contribute to differences in volume regulation between men and women.

# Introduction

The renin-angiotensin-aldosterone system (RAAS) is a main regulatory system of volume homeostasis and blood pressure. Aldosterone secretion induces sodium and water retention in the distal tubules of the kidneys, and is stimulated by angiotensin II (ang II) and a high plasma potassium concentration.

Differences in the RAAS between men and women have been described[1-5]. Higher aldosterone levels have been reported in men, both in normotensive and in hypertensive subjects[5,6]. However, it is unknown whether gender differences in aldosterone levels are associated with functional consequences on volume homeostasis[1]. As the major effect of aldosterone is sodium and water retention, we hypothesize that a higher aldosterone level in men is associated with a higher extracellular volume (ECV). Furthermore, gender differences in regulation of aldosterone production are not well studied.

To study gender differences in RAAS and ECV, maintaining standardized study conditions is mandatory. RAAS hormone levels vary with sodium diet and, in women, with phase of the menstrual cycle[7]. Therefore, in this study we investigated gender differences in aldosterone levels, ECV and blood pressure during a low and high sodium intake, in a steady state and standardized for menstrual cycle. Furthermore, we studied gender differences in the adrenal response to ang II infusion in these standardized conditions, during both sodium intakes.

# Methods

*Study population*

The study population consisted of 36 healthy, Caucasian subjects (women, *n*=18; men, *n*=18) which took part in the GRECO program, which is an ongoing study program on renal hemodynamic studies in different populations (healthy and chronic kidney disease patients) with standardized measurements and harmonized protocols for different subsequent studies, allowing combined analyses of the different sub-studies. The women were studied in the RETAP sub-study and compared with men from the Gene-Environment sub study [8,9]. All subjects were non-smokers and normotensive, having a sitting systolic blood pressure < 140 mmHg and diastolic blood pressure < 90 mmHg measured by Dinamap, and were not treated with an antihypertensive drug. Their medical history revealed no significant diseases. Subjects with obesity (BMI >30 kg/m$^2$ at screening) were excluded. Physical examination and electrocardiography did not reveal any abnormalities. None of the women were users of oral contraceptive medication, or were pregnant. Both studies were approved by the local medical ethical committee (METc number: RETAP study 2010/294, www.trialregister.nl; trial registration number: 2635, Gene-Environment study 2001/012) and all subjects gave written informed consent in accordance with the Declaration of Helsinki.

*Study protocol*

In both women and men, a standardized cross-over protocol was performed as described earlier[9,10], which consisted of two one-week periods: in random order a 7-day period on a low sodium diet (LS; aim: 50 mmol $Na^+$/day), and a 7-day period on a high sodium diet (HS; aim: 200 mmol $Na^+$/day), with a stable potassium intake. This was achieved by dietary counseling. For assessment of dietary compliance and the achievement of a stable sodium balance, 24h urine was collected at day 3 and day 6 during each period. In men the study periods were done consecutively, and in women these periods were divided by one menstrual cycle, to avoid the influence of momentarily sex hormones to aldosterone levels and ang II responsiveness[1,11]. At day 7 of both study periods, during which all women were in the mid-follicular phase (day 7±2 of menstrual cycle), the subjects reported at the research unit at 8am after an overnight fast. Body weight, length and waist-to-hip ratio were measured at the start of this day. An intravenous cannula was inserted into each forearm, one for drawing blood samples, the other for infusion of ang II. Subjects received standardized meals and fluids during the day, with sodium intake adjusted to the prescribed diet. To ensure sufficient urine output, infusion of 250 mL/h of 5% glucose was administered and every hour 250 mL of oral fluids were provided. Baseline values for blood pressure were obtained from 10am to 12am. Between 12am and 3pm ang II (Clinalfa, Merck Biosciences AG, Läufelfingen, Switzerland) was administered intravenously, at a constant rate in doses of 0.3, 1 and 3 ng/kg/min each during 1h.

Blood pressure and heart rate were measured with an automated sphygmomanometer (Dinamap; GE Medical Systems, Milwaukee, Wisconsin, USA) at 15-min intervals. Subjects were seated in a quiet room in a semi-supine position, with their arm in resting position. During ang II infusions, blood pressure was measured at 5-min intervals. Appropriate blood pressure cuff was determined on the basis of arm circumference.

ECV was measured as the distribution volume of 125I-iothalamate during steady state, as described in more detail previously[12]. This was performed before ang II infusions. Briefly, the distribution volume of 125I-iothalamate is calculated from the plasma level of 125I-iothalamate divided by the total amount of 125I-iothalamate in the body, which equals the amount infused minus the amount excreted. It is calculated as sum(I × V) + Bolus – sum(U × V)/P, and expressed as ECV/body surface area (BSA), i.e., l/1.73 $m^2$ BSA. BSA was calculated according to the DuBois-DuBois formula[13].

*Sample collection and analytical methods*

Blood samples were drawn at baseline and after each hour of ang II infusion. Blood for measuring plasma aldosterone and renin was collected in precooled tubes and immediately centrifuged at 4°C for 10min (3000 rpm). Plasma was subsequently stored at -80°C until analysis. Aldosterone was measured with a commercially available radioimmunoassay kit (Diagnostic Products Corporation, Los Angeles, California, USA). Active plasma renin concentration (APRC) was measured with a radioimmunoassay that detects the amount of angiotensin I produced per hour in the presence of excess exogenous angiotensinogen as described previously[14] (nanograms of angiotensin I produced per liter of plasma per hour; CisBio International, France). Longitudinal quality controls were run in all assays in order to validate the results over time. The level of urinary sodium, potassium and urea were determined from the 24h-urine collections of the subjects, and assessed by the use of an automated clinical chemistry analyzer (Roche Modular Basel).

*Statistical analysis*

Statistical analysis was performed using SPSS for Windows (Version 22.0). Data were tested for normality using histograms and the Kolmogorov-Smirnov test for normal distribution. Parametric data are presented as mean ± standard deviation (SD) in text, tables and figures, and analyzed using the Student's t-test or paired t-test. Nonparametric data are presented as median (25th-75th percentile) and analyzed using the Mann Whitney-U test or Wilcoxon Signed rank test. Gender differences in ECV and aldosterone during a low sodium diet and high sodium diet were analyzed by generalized estimating equations (GEE) analysis. Gender differences in aldosterone response were determined using GEE analysis. Statistical significance was accepted at $p<0.05$. The association between plasma aldosterone and ECV was tested using linear univariate regression analysis. For this end, plasma aldosterone was log transformed to achieve normal distribution.

# RESULTS

*Baseline characteristics and urinary and blood parameters*

The baseline characteristics of the two groups are presented in Table 1. There were no significant differences in age, waist-to-hip ratio and BMI. Height and BSA were, as expected, significantly higher in men. Urinary albumin excretion was normal in all subjects, and did not differ between men and women (data not shown). Blood and urinary parameters during the different sodium intakes are shown in Table 2. Systolic blood pressure was higher in men during both sodium intakes (LS: 122 ± 10 vs 110 ± 9 mmHg, P=0.001; HS: 124 ± 12 vs 115 ± 8 mmHg, P=0.011). Diastolic blood pressure was higher in men during a low sodium diet (72 ± 7 vs 67 ± 7, P=0.039), but not significantly different during a high sodium diet (73 ± 8 vs 71 ± 8 mmHg, P=0.474). Urinary sodium excretion and urinary potassium excretion were equal between both groups, which reflects comparable sodium and potassium intakes during the respective dietary weeks.

**Table 1.** Characteristics of subjects

| Characteristic | Women (*n*=18) | Men (n=18) | P |
|---|---|---|---|
| Age, years | 36 ± 5 | 31 ± 11 | 0.092 |
| Waist-to-hip ratio | 0.83 ± 0.04 | 0.85 ± 0.08 | 0.397 |
| Height, cm | 171 ± 5 | 184 ± 6 | **<0.001** |
| BMI, kg/m$^2$ | 23.2 ± 2.7 | 23.2 ± 2.2 | 0.969 |
| BSA, m$^2$ | 1.79 ± 0.12 | 2.01 ± 0.12 | **<0.001** |

BMI, body mass index; BSA, body surface area; Data are presented as mean ± SD. Differences between men and women are analyzed by using Student's t-test.

*RAAS hormones, extracellular volume, and their association*

Aldosterone was significantly higher in men than in women during a high sodium diet intake (37 (24-63) ng/L vs 26 (10-34) ng/L, P=0.014). During a low sodium diet, this difference was no longer statistically significant (92 (72-145) ng/L vs 121 (77-154) ng/L, P=0.252; Table 2, fig 1A). APRC was significantly higher in women during both sodium intakes (LS: 9.5 (8.1-12.7) vs 5.6 (4.3-7.4) ng Ang-I/mL/h, P<0.001; HS: 4.0 (2.5-6.0) vs 2.8 (1.2-3.5) ng Ang-I/mL/h, P=0.024; Table 2). ECV data (scaled as ECV/1.73m$^2$ BSA) is shown in fig 1B. Men had a significantly higher ECV than women during both sodium intakes (LS: 13.3 ± 1.8 vs 16.3 ± 2.6 L/1.73m$^2$, P=0.001; HS: 14.4 ± 2.2 vs 17.4 ± 2.9 L/1.73m$^2$, P=0.002). As expected ECV was higher during high sodium intake than during low sodium intake in both men (P=0.023) and women (P=0.006). Similar results were seen when scaling ECV to lean body mass, or to weight (data not shown).

**Table 2.** Clinical parameters during low and high sodium intake

| Parameter | Women (*n*=18) | Men (*n*=18) | P |
|---|---|---|---|
| SBP HS, mmHg | 115 ± 8 | 124 ± 12 | **0.011** |
| SBP LS, mmHg | 110 ± 9# | 122 ± 10 | **0.001** |
| DBP HS, mmHg | 71 ± 8 | 73 ± 8 | 0.474 |
| DBP LS, mmHg | 67 ± 7# | 72 ± 7 | **0.039** |
| Heart rate HS, beats/min | 67 ± 8 | 57 ± 7 | **<0.001** |
| Heart rate LS, beats/min | 67 ± 8 | 60 ± 11 | **0.021** |
| Plasma potassium HS, mmol/L | 3.9 ± 0.2 | 3.9 ± 0.3 | 0.667 |
| Plasma potassium LS, mmol/L | 4.0 ± 0.2 | 4.0 ± 0.2 | 0.944 |
| Urinary sodium HS, mmol/24h | 221 ± 64 | 200 ± 70 | 0.356 |
| Urinary sodium LS, mmol/24h | 39 ± 14# | 41 ± 27# | 0.764 |
| Urinary potassium HS, mmol/24h | 80 ± 34 | 68 ± 22 | 0.215 |
| Urinary potassium LS, mmol/24h | 66 ± 21 | 76 ± 30 | 0.267 |
| Urinary creatinine HS, mmol/24h | 9.8 ± 1.5 | 15.3 ± 2.3 | **<0.001** |
| Urinary creatinine LS, mmol/24h | 9.8 ± 1.9 | 13.9 ± 2.9 | **<0.001** |
| Aldosterone HS, ng/L | 26 (10-34) | 37 (24-63) | **0.014** |
| Aldosterone LS, ng/L | 92 (72-145) # | 121 (77-154) # | 0.252 |
| APRC HS, ng Ang-I/mL/h | 4.0 (2.5-6.0) | 2.8 (1.2-3.5) | **0.024** |
| APRC LS, ng Ang-I/mL/h | 9.5 (8.1-12.7) # | 5.6 (4.3-7.4) # | **<0.001** |

HS, high sodium intake; LS, low sodium intake; SBP, systolic blood pressure; DBP, diastolic blood pressure; APRC, active plasma renin concentration. Data are presented as mean ± SD or median ($25^{th}$-$75^{th}$ percentile). Differences between men and women are analyzed by using the Student's t-test or the Mann Whitney U test. Differences between low sodium intake vs high sodium intake are tested by using a paired t-test or Wilcoxon Signed rank test. # P<0.05 LS vs HS.

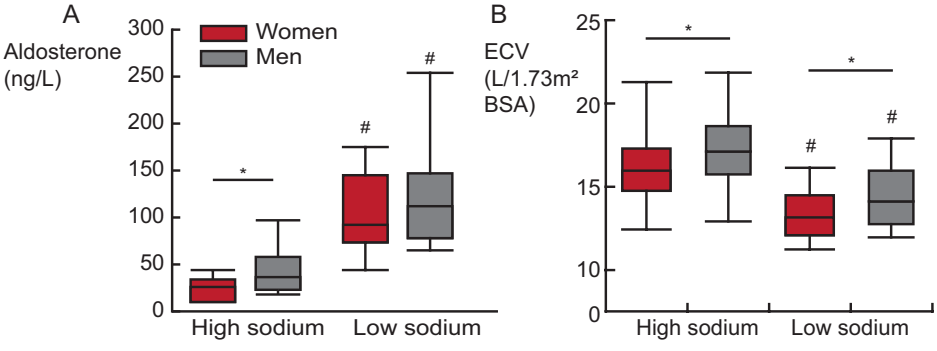


**Figure 1.** Plasma aldosterone and extracellular volume in men and women during high and low sodium intake. Median ($75^{th}$ percentile) (A) plasma aldosterone and (B) ECV during high sodium and low sodium intake in women (white boxplots), and men (grey boxplots). The whiskers represent the $10^{th}$ and $90^{th}$ percentile. BSA: body surface area. * Significantly different from women (GEE analysis), p<0.05. # Significantly different between low and high sodium intake (GEE analysis), p<0.05. The response of extracellular volume and aldosterone after the change in sodium intake was not significantly different between both groups (GEE analysis).

In the whole population, a higher plasma aldosterone was associated with a higher ECV during a high sodium diet (B=1.758, P=0.024, see figure 2). During a low sodium intake, this trend was borderline significant (B=1.526, P=0.103). When investigating this association per gender, no statistically significant correlations were found (data not shown). The extent of ECV reduction after sodium restriction was not correlated with the rise in aldosterone, or with blood pressure decline. Additionally, blood pressure reduction after sodium restriction was not statistically significantly correlated with the rise in aldosterone.

*Adrenal response to angiotensin II infusion*

To study gender differences in the regulation of aldosterone, we performed ang II infusions during a low and high sodium diet. In both men and women, the increasing doses of ang II led to a progressive increase in aldosterone levels (fig 3). In women this increase in aldosterone levels was more pronounced than in men during both sodium intakes (analysis of dose response curves by GEE analyses).

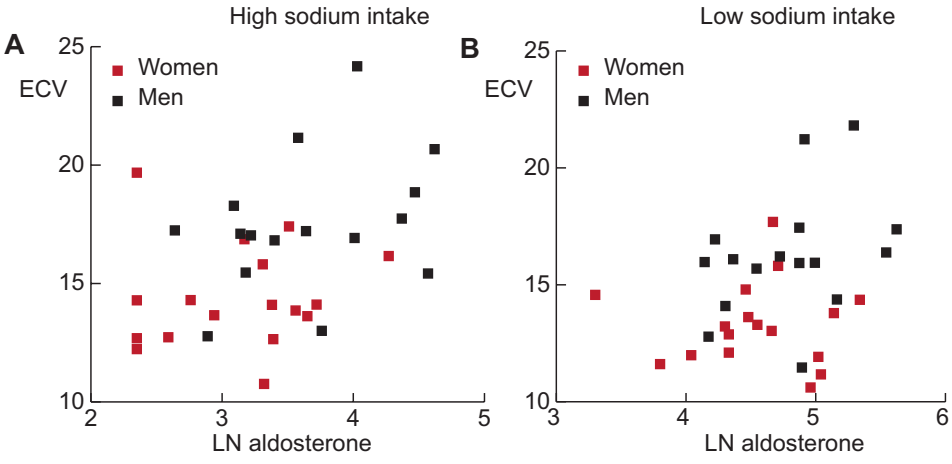


**Figure 2.** Scatterplot of distribution of extracellular volume (ECV) against plasma aldosterone during high sodium diet, and low sodium diet. (A) High sodium diet. (B) Low sodium diet. In the whole population a higher plasma aldosterone was statistically significant associated with a higher ECV, during a high sodium intake, and borderline significant during a low sodium intake.

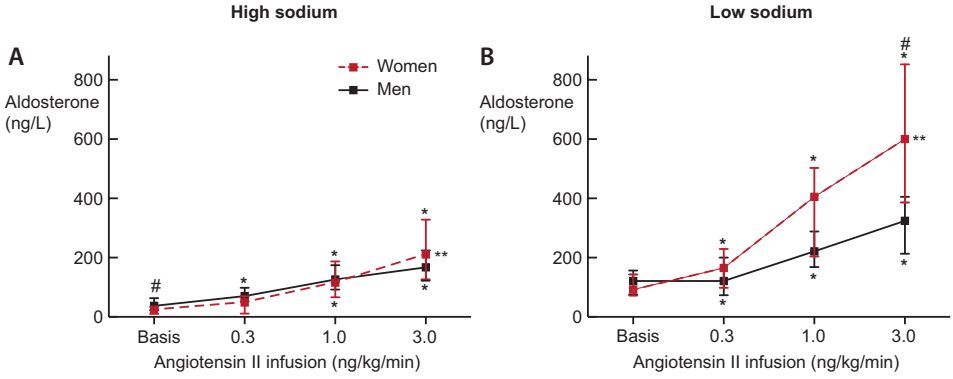


**Figure 3.** Median (with 25th-75th percentile) aldosterone concentration during angiotensin II infusion on high sodium intake and low sodium intake in women (open line) and in men (black line). (A) High sodium diet. (B) Low sodium diet. * significantly different from baseline (Mann-Whitney U test), $p<0.05$. # significantly different from women (Mann-Whitney U test), $p<0.05$. ** curves of men and women significantly different (GEE analysis, corrected for baseline values), $p<0.05$.

## Discussion

This is the first study providing a systematic comparison of aldosterone and volume status in healthy young adult men and women, under strictly standardized conditions on both a high and low sodium diet. Our data suggest that constitutive gender differences in aldosterone levels may lead to altered volume status with a higher ECV and blood pressure in men. Additionally, men have a reduced adrenal response to exogenous ang II infusion, compatible with a higher effect of endogenous ang II on adrenal aldosterone secretion[15]. Therefore, the difference in aldosterone levels might be ang II mediated.
We found a higher plasma aldosterone in men than in women. This is in accordance with earlier studies, in both healthy and hypertensive subjects[5,6]. During a low sodium diet, the difference in aldosterone between men and women did not quite reach statistical significance.

The higher aldosterone in men we report could be explained by different mechanisms. First, differences in plasma potassium concentrations could influence aldosterone levels, however these were similar in men and women. Secondly, higher levels of plasma ACTH levels could stimulate additional aldosterone secretion in men, however these were not measured in the current study. Lastly, higher circulating levels of endogenous ang II, or higher adrenal sensitivity to endogenous ang II, could contribute to the higher aldosterone levels in men. However, endogenous ang II was not measured as this is notoriously difficult to interpret, and therefore we prefer assessing endogenous ang II using infusion of exogenous ang II. Indeed we found that the adrenal responses to exogenous ang II infusion were less pronounced in men, on both sodium intakes. A lower adrenal response to exogenous ang II could be due to several factors related to greater endogenous ang II activity, such as an increased tissue concentration of endogenous angiotensin II or increased density of the angiotensin II receptor[15]. Therefore, the reduced adrenal response to ang II infusion we found in men, suggests endogenous ang II facilitates the higher aldosterone levels in men. We previously reported on gender differences in ang II response – respectively of blood pressure, inversely to the current manuscript – with a larger response in men during high sodium[16]. This is in line with the reciprocal response to altered endogenous ang II status between ang II sensitivity of the vascular bed and that of the adrenal gland[15].

Furthermore, we are the first to demonstrate gender differences in volume status under well-controlled conditions. We found that ECV was higher in men, both during a high and a low sodium diet. This finding was consistent when normalizing ECV to other body dimensions (i.e. length and lean body mass), marking the robustness of our data. This is in line with the results of Peters *et al*. who found a higher ECV (scaled to BSA) in men,

in a large cohort study of healthy prospective kidney donors[17]. However, in their study sodium status was not standardized, and the ECV difference did not persist when scaled to other body dimensions, or when corrected for potassium intake. Our data demonstrate an effect of sodium intake in ECV, with a rise in ECV during a high sodium diet. This shows that it is relevant to account for sodium intake when interpreting ECV.

We found a higher systolic blood pressure in men, under well controlled conditions. Heart rate was higher in women than in men, which is in line with known literature in healthy young adults[18]. In the hypertensive population, it has been well-established that blood pressure is higher in men than in pre-menopausal women[19,20]. Here, we show that in normotensive subjects this is true as well, which is in line with previous studies[21,22]. This might be mediated through gonadal hormones; testosterone levels in men might increase systolic blood pressure (SBP) [23], while estrogen levels in women might protect against high SBP[19]. It has also been suggested that gender differences in sympathetic regulation of the cardiovascular system lead to differences in SBP[24]. Alternatively, as we found that men have higher aldosterone levels and higher ECV, excess volume and sodium retention elicited through aldosterone might lead to higher SBP. Indeed we found an association between higher aldosterone and higher ECV. However, this association was not found in women and men separately, and was only borderline significant during a low sodium diet. While our data support the hypothesis that aldosterone causes a higher SBP in men through volume retention, intervention with an aldosterone antagonist such as spironolactone or eplerenone would provide further evidence.

We found that SBP decline after sodium restriction was subtle, and in men did not reach statistical significance. This demonstrates an intact blood pressure homeostasis in non-sodium sensitive normotensive young adults. The absence of a visible correlation between ECV decline and SBP decline after sodium restriction further illustrates the intact feedback loop to maintain BP despite volume loss.

Our study has limitations. First, our study shows an association between gender differences in aldosterone and ECV, but cannot provide proof of the causality of this association. Second, we studied pre-menopausal women in the mid-follicular phase, caution is warranted when extrapolating our findings. Aldosterone levels and ECV are influenced by phase of the menstrual cycle, and, importantly, by menopause[25,26]. It has been shown that after menopause the gender differences in aldosterone levels and in blood pressure disappear[27-30]. Furthermore, we found significantly lower ARPC in men than in women, irrespective of sodium intake. This is in contrast with earlier studies, which describe a lower plasma renin in premenopausal women than in men[31,32]. This could not be explained through phase of the menstrual cycle, as renin levels were found to be lower

during the follicular phase than during the luteal phase[31]. As we measured APRC in two different sub-studies of the GRECO-cohort, and the measurements were performed several years apart, these results should be interpreted with caution.

In conclusion, men have a higher aldosterone, ECV and SBP than women. Furthermore, the adrenal response to ang II infusion is less pronounced in men, suggesting a higher contribution of endogenous ang II to adrenal aldosterone secretion. Taken together, this well controlled physiological study gives in-depth data on possible mechanisms in which gender difference in aldosterone could lead to a higher ECV and blood pressure in men.

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# CHAPTER 9

## Renoprotective RAAS inhibition does not affect the association between worse renal function and higher plasma aldosterone levels

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# Abstract

**Background** Aldosterone is elevated in chronic kidney disease (CKD) and may be involved in hypertension. Surprisingly, the determinants of the plasma aldosterone concentration (PAC) and its role in hypertension are not well studied in CKD. Therefore, we studied the determinants of aldosterone and its association with blood pressure in CKD patients. We also studied this during renin-angiotensin-aldosterone system inhibition (RAASi) to establish clinical relevance, as RAASi is the treatment of choice in CKD with albuminuria.

**Methods** We performed a post-hoc analysis on data from a randomized controlled double blind cross-over trial in non-diabetic CKD patients ($n$=33, creatinine clearance (CrCl) 85 (75-95) ml/min, proteinuria 3.2 (2.5-4.0) g/day). Patients were treated with losartan 100 mg (ARB), and ARB + hydrochlorothiazide 25 mg (HCT), during both a regular (200 ± 10 mmol $Na^+$/day) and low (89 ± 8 mmol $Na^+$/day) dietary sodium intake, in 6-week study periods. PAC data at the end of each study period were analyzed. The association between PAC and blood pressure was analyzed continuously, and according to PAC above or below the median.

**Results** Lower CrCl was correlated with higher PAC during placebo as well as during ARB (β=-1.213, P=0.008 and β=-1.090, P=0.010). Higher PAC was not explained by high renin, illustrated by a comparable association between CrCl and the aldosterone-to-renin ratio. The association between lower CrCl and higher PAC was also found in a second study with single RAASi with ACE inhibition (ACEi; lisinopril 40mg/day), and dual RAASi (lisinopril 40mg/day + valsartan 320 mg/day). Higher PAC was associated with a higher systolic blood pressure (P=0.010) during different study periods. Only during maximal treatment with ARB + HCT + dietary sodium restriction, blood pressure was no longer different in subjects with a PAC above and below the median.

**Conclusions** In CKD patients with a standardized regular sodium intake, worse renal function is associated with a higher aldosterone, untreated and during RAASi with either ARB, ACEi, or both. Furthermore, higher aldosterone is associated with higher blood pressure, which can be treated with the combination of RAASi, HCT and dietary sodium restriction.

# Background

Aldosterone is involved in sodium and volume homeostasis, and consequently regulation of extracellular volume and blood pressure. It has a main role in the homeostatic response to volume depletion, where higher aldosterone levels contribute to renal retention of sodium and water, and followingly restoration of the extracellular volume. In chronic kidney disease (CKD), derangement of volume status and hypertension are common. Several studies indicate an inverse association between plasma aldosterone levels and creatinine clearance[1-4].

Inappropriately high aldosterone levels in CKD have been suggested to contribute to CKD-associated hypertension, as well as to progressive kidney damage by direct profibrotic effects of aldosterone[5-7]. Inhibition of the renin-angiotensin-aldosterone system is a cornerstone of therapy in CKD, for treatment of hypertension and proteinuria[8]. Whether RAAS inhibition (RAASi) ameliorates the high aldosterone levels associated with CKD, however, has not been systemically assessed.

Therefore, we aim to investigate, first, the determinants of the plasma aldosterone concentration (PAC) and aldosterone-to-renin ratio (ARR) in non-diabetic CKD, both without and with RAAS inhibition (RAASi). Second, we aim to investigate whether in CKD, high aldosterone is associated with blood pressure.

To this purpose, we studied the association between PAC, ARR and renal function during different treatment conditions in a previously performed randomized controlled trial. Here, ARB (losartan 100 mg/day) and placebo were compared in combination with dietary sodium restriction and HCT (hydrochlorothiazide 25 mg/day) in non-diabetic proteinuric CKD patients[9]. In this analysis we found that during ARB, renal function was negatively correlated with PAC, similarly as during placebo. To provide independent confirmation of the lack of efficacy of RAASi on the association between lower renal function and higher PAC, we analysed the association between PAC, ARR and creatinine clearance in a second study with comparable design. This second study had study periods of single RAASi with ACE inhibition (ACEi), and dual RAASi with ACEi + ARB, both during a standardized regular and low sodium intake[10].

# Methods

*Patients and study design*

This is a post-hoc analysis of a previously performed randomized controlled cross-over trial, the protocol is described in detail elsewhere[9]. For all clinical experimentation, approval was obtained from the Institutional Review Board (Medical Ethics Committee of the University Medical Centre Groningen) with oversight authority for the protection of human research subjects. The study was performed according to the Declaration of Helsinki of 1975, as revised in 2000. All participants gave written informed consent before study-related procedures were performed. In short, patients had stable proteinuria due to non-diabetic CKD, were middle aged, and had stable creatinine clearance (>30 mL/min, <6 mL/min/year decline). Patients were randomized to a low sodium diet (target sodium intake 50 mmol $Na^+$/day; approximately 1200 mg $Na^+$/day or 3 g NaCl/day) or a regular sodium diet (target sodium intake 200 mmol $Na^+$/day; 4800 mg $Na^+$/day or 12 g NaCl/day). Dietary counselling was aimed at avoiding salty foods while keeping other dietary habits unchanged. Patients remained on the assigned diet for 18 weeks, consisting of three consecutive 6-week study periods. Placebo was always as the first treatment, followed by ARB (losartan 100mg/day), and ARB + HCT (losartan 100mg/day + hydrochlorothiazide 25mg/day), where ARB and ARB + HCT were given in random order. After 18 weeks, the patients changed their diet and the three 6-week periods were repeated in the same order as the first three 6-week periods. Therefore, in total there were 4 possible treatment sequences. Additional antihypertensive drugs were allowed if required; these were kept constant during the study.

*Independent confirmation: no effect RAASi on the association of creatinine clearance and PAC.*

We assessed the effect of single RAASi with ACEi vs dual RAASi with ACEi+ARB, respectively, on the association between PAC, ARR and creatinine clearance in an independent second study, with a comparable cross-over design during different sodium intakes. The protocol is described in detail elsewhere[10]. Patients had comparable non-diabetic CKD with stable proteinuria and creatinine clearance (>30mL/min, <6 mL/min/year decline) In this trial ($n$=45) the 6-week study periods consisted of single RAASi (lisinopril 40mg/day) and dual RAASi (lisinopril 40mg/day + valsartan 320 mg/day), both during a regular sodium diet (target 200 mmol $Na^+$/day) and a low sodium diet (target 50 mmol $Na^+$/day), in random order. As 7 patients participated in both trials, we excluded them from the second analysis, to avoid duplication of the results.

*Measurements and calculations*

Proteinuria was measured by the pyrogallol red-molybdate method in 24-hour urine samples. Blood pressure was determined at one minute intervals with an automatic device (Dinamap, GE Medical Systems, Milwaukee, WI, USA) with the patient in a supine position. After 15 minutes of measurements, we used the mean of the last three readings for further analyses. Dietary sodium intake was assessed from 24-hour urinary sodium excretion. We calculated creatinine clearance from creatinine concentrations in plasma and in 24-hour urine samples. Peripheral blood was drawn by venipuncture, and aliquots from serum were stored (-80°C) until plasma aldosterone concentration (PAC) and active plasma renin concentration (APRC) analysis. Samples were taken in a standardized manner, with the patient in a supine position, and were directly put on ice. Aldosterone was measured with a commercially available RIA kit (Diagnostic Products Corp., Los Angeles, CA). Plasma renin activity was measured as described previously with a RIA that detects the amount of angiotensin I produced per hour in the presence of excess angiotensinogen (nanograms of angiotensin I produced per millilitre of plasma per hour) [11]. This assay measures the enzymatic activity of active plasma renin in the presence of an excess of its (exogenous) substrate.

In the second study, renin was determined using direct renin measurements, in this respect, data regarding the aldosterone-to-renin ratio in both studies could not be pooled. The remaining measurements and calculations in the second study used for the independent analysis were performed equally[10].

*Statistics*

All 33 patients were included in our data analysis, and are presented here. Skewed variables were natural log (LN) transformed in order to achieve a normal distribution for linear regression and linear mixed model analyses. Data is given as mean with standard deviation when normally distributed, and geometric mean with 95% confidence interval when data is skewed. SPSS 22.0 for Windows was used for all analyses.

To assess the determinants of PAC and ARR, linear regression analyses were performed. The log transformed PAC and ARR were used as the dependent factors. Analysed variables are: age, gender, body mass index, serum sodium, 24-hour urinary sodium excretion, serum potassium, 24-hour urinary potassium excretion, log transformed plasma renin activity, log transformed proteinuria and log transformed creatinine clearance. This analysis was equally performed in data from the second study.

Treatment effects were determined using linear mixed model analyses, with Sidak post-hoc analyses to localize the differences. Statistical significance was assumed at the 5% level of probability. The covariance structure for all mixed model analyses was based on Akaike's information criterion. The association between PAC, and systolic blood pressure (SBP) was studied using linear mixed model analyses. First the association between aldosterone and SBP, and possible confounders (gender, age, creatinine clearance), were tested univariately. In univariate analysis, SBP was the dependent variable, and sodium diet, ARB, HCT, their interaction (sodium diet x ARB x HCT), and log transformed PAC (or gender, age, creatinine clearance) were fixed factors. Subjects was used as a random factor. Covariates with a $P<0.100$ were included in the multivariate mixed model.

To ensure there were no carryover effects from the different treatment regimens, we performed linear mixed model analyses. Log transformed PAC was the dependent factor, and treatment and sequence as well as their interaction (treatment x sequence) were fixed factors. We did similar analyses with log transformed ARR, and systolic blood pressure as the dependent factors. Carry-over effects were not detected (treatment x sequence was not a statistically significant predictor), and for the sake of conciseness these data are not shown.

# Results

*Baseline characteristics and responses to treatment*

Patient characteristics and adherence to the program have been described previously[9], (see also supplementary table 1). In total 33 participants have completed the study. During regular dietary sodium intake, mean 24-hour urinary sodium excretion was 197 ± 63 mmol $Na^+$/day (approximately 4600 mg $Na^+$/day or 12 g NaCl/day), and during low dietary sodium intake (LS) this was 92 ± 46 mmol $Na^+$/day (approximately 2100 mg $Na^+$/day or 5 g NaCl/day; Table 1). Systolic blood pressure dropped stepwise with the lowest value on ARB + HCT + LS, whereas diastolic blood pressure was lowest on ARB + HCT on either sodium intake. Creatinine clearance fell significantly after sodium restriction during both ARB and ARB + HCT treatment. Proteinuria declined after each additional treatment step, with the lowest value in ARB + HCT + LS.

*PAC and ARR during different treatment conditions*

PAC did not change during ARB as compared to placebo, neither during the regular nor during low sodium intake (P=0.9 and P>0.999 respectively, Table 1). However, the ARR declined significantly during ARB in both sodium intakes, illustrating pharmacological inhibition of the RAAS by ARB. The addition of HCT to ARB increased PAC in both sodium intakes (P<0.01 and P=0.01), illustrating reduction of extracellular fluid volume. Dietary sodium restriction was associated with a higher PAC during ARB and ARB + HCT, but not during placebo treatment.

*Determinants of PAC and ARR, during placebo and single RAASi with ARB*

As anticipated, during placebo, creatinine clearance was negatively and significantly associated with PAC (β=-1.213, P=0.008; Table 2). During ARB, the negative correlation between creatinine clearance and PAC was similarly present (Figure 1). Neither plasma renin activity (placebo β=0.274, P=0.29; ARB β=0.171, P=0.30), nor serum potassium (placebo β=0.346, P=0.42; ARB β=0.011, P=0.9) were significant determinants of PAC in either treatment condition. During low dietary sodium intake results were virtually similar. In multivariate analysis, when adjusting for age and gender, creatinine clearance remained the only significant predictor of PAC in both treatment conditions (supplementary Table 2).

Predictors of the aldosterone-to-renin ratio (ARR) were similar to those of PAC (Table 2, figure 1). Again, as anticipated, during placebo treatment, creatinine clearance was negatively significantly correlated with ARR (β=-1.215, P=0.02). During ARB, the association was similarly present (β=-1.475, P=0.01), although the regression line was shifted downwards, illustrating pharmacological inhibition of the RAAS by ARB treatment (figure 1). During low dietary sodium intake results were virtually similar.

**Table 1.** Clinical and biochemical parameters during different study periods

| | | Placebo | ARB | ARB+HCT |
|---|---|---|---|---|
| Systolic blood pressure (mmHg) | RS | 143 (4) | 135 (3)† | 125 (3)†‡ |
| | LS | 136 (3)* | 128 (3)*† | 121 (2)†‡ |
| Diastolic blood pressure (mmHg) | RS | 86 (2) | 80 (2)† | 75 (1)†‡ |
| | LS | 83 (1)* | 78 (1)† | 74 (1)†‡ |
| Plasma potassium concentration (mmol/l) | RS | 4.3 ± 0.1 | 4.4 ± 0.1 | 4.0 ± 0.1†‡ |
| | LS | 4.3 ± 0.1 | 4.5 ± 0.1† | 4.0 ± 0.1†‡ |
| 24h sodium excretion (mmol/day) | RS | 200 ± 10 | 197 ± 11 | 193 ± 11 |
| | LS | 89 ± 10* | 92 ± 8* | 93 ± 8* |
| Creatinine clearance (ml/min) | RS | 85 (73-98) | 90 (77-106) | 81 (69-95)‡ |
| | LS | 80 (67-94) | 79 (67-93)* | 72 (61-85)* |
| Proteinuria (g/day) | RS | 3.4 (2.6-4.3) | 2.3 (1.8-3.0)† | 1.3 (1.0-1.7)†‡ |
| | LS | 2.3 (1.7-3.1)* | 1.3 (0.9-1.7)†* | 0.9 (0.6-1.2)*†‡ |
| Active plasma renin concentration (ng AI/ml x h) | RS | 15 (12-18) | 33 (23-45)† | 64 (47-87)†‡ |
| | LS | 19 (15-23) | 51 (38-70)*† | 130 (103-163)*†‡ |
| Plasma aldosterone concentration (ng/l) | RS | 66 (48-91) | 57 (41-79) | 96 (74-125)‡ |
| | LS | 106 (73-152) | 113 (86-149)* | 164 (129-207)*†‡ |
| Aldosterone-to-renin ratio (ng/ng AI × h) | RS | 19 (13-26) | 7 (4-11)† | 6 (4-9)† |
| | LS | 22 (14-32) | 9 (6-13)† | 5 (4-7)†‡ |

* = P<0.05 vs regular sodium on same treatment (effect of LS). † = P<0.05 vs placebo on same sodium diet. ‡ = P<0.05 vs ARB on same sodium diet (effect of HCT). ARB: angiotensin receptor blocker (losartan 100mg/day). HCT: hydrochlorothiazide (25mg/day)..

*Second study. Association between CrCL and PAC during single RAASi with ACEi and dual RAASi.* In a second study, we assessed the effect of single RAASi with ACEi (lisinopril 40mg/day) and dual RAASi (lisinopril 40mg/day + valsartan 320mg/day) on the negative association between creatinine clearance and PAC and ARR during a standardized regular sodium intake[10]. Patient characteristics are shown in supplementary Table 1. The plasma aldosterone concentration during single RAASi with lisinopril was 60 (73-89) ng/L, and did not differ from the PAC in the original study during single RAASi with losartan (P=0.543). During dual RAASi the PAC did not change (71 (59-86) ng/L, P=0.993). Also in this second study, creatinine clearance was significantly and negatively correlated with PAC during single RAASi, albeit with lisinopril (β=-0.646, P<0.003). With ARR it did not quite reach statistical significance (β=-1.019, P=0.07). During dual RAASi treatment with lisinopril and valsartan, creatinine clearance was significantly and negatively correlated with both PAC and ARR (β=-0.805, P=<0.001 and β=-2.020, P=0.005 respectively).

**Table 2.** Determinants of the plasma aldosterone concentration and aldosterone-to-renin ratio during placebo and during ARB, during a regular sodium intake

| | Plasma aldosterone concentration | | | | Aldosterone-to-renin ratio | | | |
|---|---|---|---|---|---|---|---|---|
| | Placebo | | ARB | | Placebo | | ARB | |
| | B | P-value | B | P-value | B | P-value | B | P-value |
| Age (years) | 0.011 | 0.41 | 0.006 | 0.67 | 0.027 | 0.06 | 0.350 | 0.049 |
| Gender (women) | -0.030 | 0.94 | 0.492 | 0.17 | 0.494 | 0.22 | 0.760 | 0.11 |
| BMI baseline (kg/m²) | 0.009 | 0.81 | -0.009 | 0.80 | 0.015 | 0.70 | 0.029 | 0.56 |
| Serum sodium (mmol/l) | 0.041 | 0.53 | -0.086 | 0.13 | 0.034 | 0.64 | 0.260 | 0.74 |
| 24 hour urinary sodium excretion (mmol/day) | 0.001 | 0.85 | -0.002 | 0.35 | <0.001 | 0.93 | -0.003 | 0.38 |
| Serum potassium (mmol/l) | 0.346 | 0.42 | 0.011 | 0.98 | 0.377 | 0.42 | 0.355 | 0.61 |
| 24 hour urinary potassium excretion (mmol/day) | -0.001 | 0.88 | -0.003 | 0.60 | <-0.001 | >0.99 | -0.004 | 0.64 |
| LN Proteinuria (g/day) | 0.078 | 0.75 | -0.135 | 0.58 | 0.003 | 0.99 | 0.075 | 0.82 |
| LN Creatinine clearance (ml/min) | -1.213 | 0.008 | -1.090 | 0.01 | -1.215 | 0.02 | -1.475 | 0.009 |
| LN Active plasma renin concentration (ng AI/ml x h) | 0.274 | 0.29 | 0.171 | 0.30 | | | | |

ARB: angiotensin receptor blocker (losartan 100mg/day), LN: natural logarithm.

*Aldosterone and blood pressure*

To study the association between PAC and systolic blood pressure (SBP) in different treatment conditions, we performed linear mixed model analyses. Sodium diet ARB, HCT, gender and log transformed aldosterone were significant predictors of SBP (Table 3), demonstrating an association between PAC and SBP, which was independent of creatinine clearance, age, and gender.

To visualize the association between PAC and SBP, we dichotomized the group in subjects with a baseline PAC above the median and a baseline PAC below the median, also shown in figure 2. During placebo, SBP was significantly higher in the high PAC group than in the low PAC group (157 ± 25 mmHg vs 130 ± 13; P=0.001). This difference was also seen during placebo + LS (146 ± 17 vs 126 ± 12; P=0.001), ARB (143 ± 20 vs 125 ± 12; P=0.005), during ARB + LS (136 ± 14 vs 119 ± 9; P<0.001) and during ARB + HCT (132 ± 17 vs 117 ± 9; P=0.004). Only during the most extensive treatment regimen of ARB + HCT + LS, there was no statistical significant SBP difference between the groups, while the mean blood pressure is still higher is patients with a high PAC (126 ± 13 vs 117 ± 14; P=0.05).

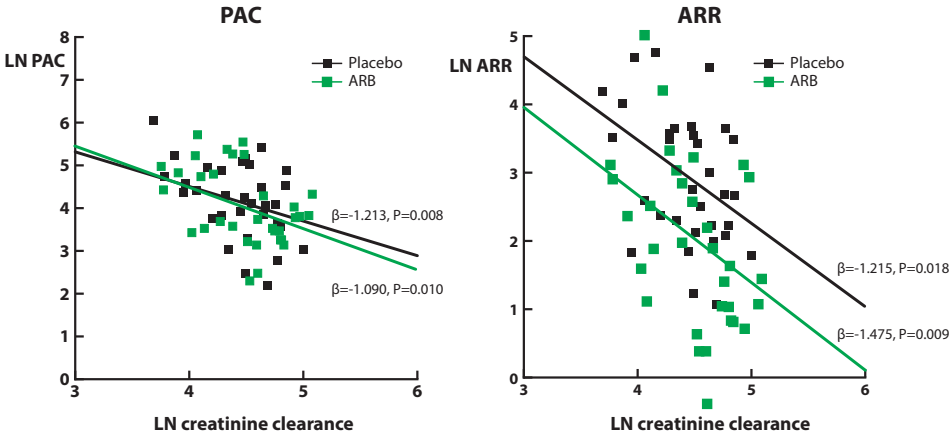


**Figure 1.** Correlation between creatinine clearance and the plasma aldosterone concentration (left panel), and aldosterone-to-renin ratio (right panel) during placebo and ARB treatment, during a regular sodium intake. Creatinine clearance is significantly and negatively correlated with PAC, and this correlation is similar during placebo and ARB treatment. ARR is similarly, and negatively correlated with creatinine clearance. During RAASi the regression line is parallel and shifted downwards. PAC: plasma aldosterone concentration; ARR: aldosterone-to-renin ratio; ARB: angiotensin receptor blocker (losartan 100mg/day).

**Table 3.** Linear mixed model analysis on the association between different predictors and the systolic blood pressure

| Parameter | Parameter estimate | P-value |
|---|---|---|
| Low sodium diet | -7.753 | <0.001 |
| ARB | -8.385 | <0.001 |
| HCT | -10.537 | 0.008 |
| Gender (women) | -7.705 | 0.01 |
| Age (years) | 0.159 | 0.16 |
| LN Creatinine Clearance (ml/min) | -5.513 | 0.14 |
| LN Aldosterone (ng/l) | 6.477 | <0.001 |

ARB: losartan 100mg/day, HCT: hydrochlorothiazide 25mg/day, PAC: plasma aldosterone concentration, LN: natural logarithm.

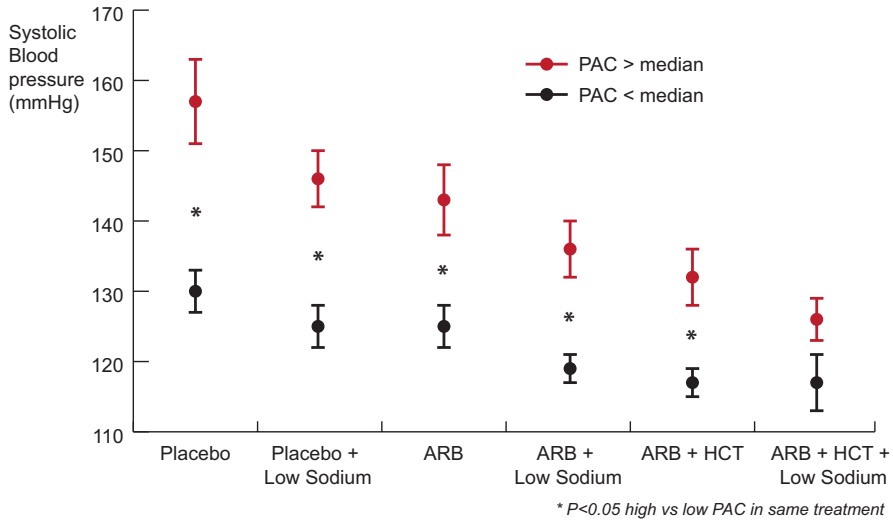


**Figure 2.** Systolic blood pressure during different treatment conditions in patients with a PAC above the median, and a PAC below the median. SBP is higher in the high PAC group in all treatment conditions, but the difference tends to decrease when treatment was intensified. Error bars represent standard error of the mean. SBP: systolic blood pressure; PAC: plasma aldosterone concentration; ARB: angiotensin receptor blocker (losartan 100mg/day); LS: dietary sodium restriction; HCT: hydrochlorothiazide (25mg/day).

## Discussion

In this study, we found that pharmacological effective RAASi did not affect the correlation between worse renal function and higher aldosterone. This was true for single RAASi with either ARB or ACEi, as well as for dual RAASi. This indicates that the association between creatinine clearance and aldosterone levels is not susceptible to RAASi, not even with dual inhibition. Furthermore, higher PAC was associated with a higher systolic blood pressure, independent of age, gender and creatinine clearance. During stepwise treatment with losartan, hydrochlorothiazide and dietary sodium restriction this difference decreased to the point where this difference was no longer statistically significant at the final treatment step (losartan, hydrochlorothiazide and a low sodium diet).

The association between lower creatinine clearance and higher PAC and ARR we observed is consistent with older data[1]. However, the older data were obtained before the era of RAASi, and did not control for sodium status. The authors reported that in patients with reduced creatinine clearance of more than 50% of normal, PAC was high while serum potassium and APRC were within normal values. Our data are consistent, and extend these findings by demonstrating that the association holds during RAASi with either ARB or ACEi. This is relevant to show, as RAASi is the treatment of choice for CKD with albuminuria. In line, Hannemann *et al*. describe an inverse association between renal function and aldosterone in a large general population cohort uncontrolled for sodium status [3]. McQuarrie *et al*. however, found that sodium status, and not renal function determined urinary mineralocorticoid excretion in CKD [12]. In our study sodium intake was standardized, which allowed to examine the effect of renal function and RAASi on PAC.

To our knowledge, the association we describe between high aldosterone and blood pressure has not been described before in CKD patients under RAASi. These data suggest that treatment with MRA is efficacious to treat hypertension in CKD. Indeed, a meta-analysis of patients with mild or moderate CKD has shown that intervention with a mineralocorticoid receptor antagonist (MRA) as adjunct to RAASi reduces blood pressure [13]. In resistant hypertensive patients without CKD, the association between high aldosterone and volume expansion has been previously established [14]. The recent PATHWAY study has shown that in treatment-resistant hypertension spironolactone as an adjunct treatment is more efficacious in lowering blood pressure than doxazosin or bisoprolol[15]. However, in this study the blood pressure lowering effect of MRA was correlated with baseline plasma renin, but not plasma aldosterone levels. In addition, in obesity, which is a different pathophysiological condition where aldosterone levels are inappropriately increased, aldosterone has also been shown to be associated with

sodium-sensitive hypertension[16-19]. We show for the first time that also in CKD, high aldosterone levels are associated with higher blood pressure, even despite RAASi.

The mechanism underlying the consistent association between lower renal function and high PAC during RAASi is unknown. The parallel association between worse renal function and ARR, and the persistence of both associations during RAASi, with either ARB or ACEi, suggest that the higher aldosterone with lower renal function is not due to renin activation. This is further supported by the persistence during dual RAASi, which makes it unlikely that the association persists due insufficient pharmacological blockade of angiotensin II and the RAAS. Furthermore, the association between worse renal function and PAC was similarly present during ARB and ACEi, which differ in various pharmacological aspects; i.e. during ARB there is a compensatory rise in angiotensin II and increased angiotensin II type 2 receptor activation, and during ACEi bradykinin levels may increase. This renders it unlikely that pharmacologically different modes of RAASi play a role in the association between renal function and PAC. The consistent association between renal function and PAC during different modes of RAASi may suggest other mechanisms and pathways are involved, which are not susceptible to RAASi. Recent data show a link between aldosterone and the calcium-phosphate-PTH-FGF23 axis [20]. There is evidence of a correlation between increased phosphaturic hormone fibroblast growth factor 23 (FGF23) and increased aldosterone through unknown mechanisms [21,22]. Also, a bidirectional interplay between PTH and aldosterone has been suggested [23-26]. Alternatively, increased intrarenal RAAS activation might be involved. In this context, it is interesting to note that it has been shown that a putative marker for intrarenal RAAS activation, is inversely correlated with renal function in diabetic kidney disease [27]. Additionally, the link between low creatinine clearance and high aldosterone could be a compensatory response to the reduced clearance of potassium that occurs when glomerular filtration rate falls, however, as the plasma potassium concentration is very tightly regulated, this hypothesis could not be tested in this study while data regarding potassium intake in respect to potassium excretion is unknown. Another possible explanation could be that it is the other way around, so that the high PAC leads to a decline in renal function due to the pro-fibrotic effects of aldosterone [5-7]. Several studies found a high PAC and ARR to be associated with the development of CKD [28,29].

What are the implications of our study? Our data show that patients with renal function impairment, over a range from mild to moderately impaired, have high aldosterone, which is associated with higher blood pressure, even despite RAASi. Thus, aldosterone might play a role in volume derangement and hypertension, even in early CKD. One might wonder whether there is a further rise in aldosterone as renal function declines, and if so, whether this translates into hypertension. Future research is imperative to investigate

the link between creatinine clearance and high aldosterone across all spectra of renal function impairment, and what would be the implications for blood pressure treatment, in particular the role of MRA treatment on top of RAASi.

The primary limitation to our study was the post-hoc design. However, the robustness of our findings is supported by the independent study in which we found that the correlation between renal function and PAC was similarly present during RAASi with ACEi, and during dual RAASi. Furthermore, in our study the addition of MRA was not investigated.

## Conclusions

Our data show a consistent association between worse renal function and higher plasma aldosterone concentration, related to a higher ARR, during RAASi with either ARB, ACEi or both. Consequently, our data support the involvement of high aldosterone levels in hypertension in CKD with mildly impaired renal function – and show that a treatment regimen of RAASi combined with stepwise sodium intervention might be effective in correcting blood pressure.

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**Supplementary Table 1**. Patient characteristics

| | Current study (*n*=33) | Independent confirmation study (*n*=45) |
|---|---|---|
| Age (years) | 50 ± 12 | 51 ± 14 |
| Male (%) | 24 (73) | 38 (84) |
| BMI (kg/m²) | 27.5 ± 4.4 | 27.4 ± 4.1 |
| Renal diagnosis | | |
| IgA nephropathy (%) | 5 (15) | 12 (27) |
| Focal segmental glomerulosclerosis (%) | 7 (21) | 13 (29) |
| Membranous nephropathy (%) | 7 (21) | 8 (18) |
| Hypertensive nephropathy (%) | 5 (15) | 6 (13) |
| Other/inconclusive (%) | 9 (27) | 6 (13) |
| Urinary sodium excretion (mmol/24 hours) | 200 ± 57 | 173 ± 73 |
| eGFR (ml/min•1.73m²) | 60 ± 20 | 60 ± 27 |
| Creatinine clearance (ml/min) | 85 (75-95) | 68 (58-79) |
| Proteinuria (g/day) | 3.2 (2.5-4.0) | 1.6 (1.2-2.1) |

BMI, body mass index; eGFR, estimated glomerular filtration rate

**Supplementary Table 2.** Multivariate analysis on the determinants of the plasma aldosterone concentration during placebo and during losartan treatment

| **Placebo** | | Model 1 | | Model 2 | |
|---|---|---|---|---|---|
| | | β | P-value | β | P-value |
| LN Creatinine clearance (ml/min) | | -1.213 | 0.008 | -1.221 | 0.013 |
| Age (years) | | | | 0.003 | 0.795 |
| Gender (Women) | | | | -0.149 | 0.618 |
| | *$R^2$* | | *0.211* | | *0.220* |
| | *P-value* | | *0.008* | | *0.069* |

| **Losartan** | | Model 1 | | Model 2 | |
|---|---|---|---|---|---|
| | | β | P-value | β | P-value |
| LN Creatinine clearance (ml/min) | | -1.090 | 0.010 | -0.973 | 0.041 |
| Age (years) | | | | 0.004 | 0.777 |
| Gender (Women) | | | | 0.216 | 0.572 |
| | *$R^2$* | | *0.203* | | *0.212* |
| | *P-value* | | *0.010* | | *0.079* |

*LN, natural logarithm.*

# CHAPTER 10

## Lower Renal Function is Associated with Derangement of 11Beta Hydroxysteroid Dehydrogenase in Type 2 Diabetes

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# Abstract

**Context**
Derangement of 11-beta hydroxysteroid dehydrogenase type 1 and type 2 (11β-HSD1 and 11β-HSD2), which regulate intracellular cortisol production, has been suggested in both type 2 diabetes (T2D) and chronic kidney disease (CKD). However, activity of 11β-HSD enzymes in patients with T2D and CKD has never been assessed.

**Objectives**
To compare 11β-HSD activities between patients with T2D and healthy controls, and assess whether in T2D, renal function is associated with 11β-HSD activities.

**Design**
Cross-sectional analysis in the DIAbetes and LifEstyle Cohort Twente (DIALECT-1).

**Setting**
Referral center for type 2 diabetes.

**Patients**
Patient with type 2 diabetes (*n*=373, age 64 ± 9 years, 58% men, 26% of patients eGFR <60 ml/min•1.73m2) and healthy controls (*n*=275, age 53 ± 11 years, 48% men).

**Mean outcome measure**
We measured cortisol, cortisone and metabolites (tetrahydrocortisol (THF), alloTHF (aTHF) and tetrahydrocortisone (THE)) in 24h urine samples. Whole body 11β-HSD and 11β-HSD2 activities were calculated as the urinary (THF+aTHF)/THE and cortisol/cortisone ratios respectively.

**Results**
Patient with T2D had a higher (THF+aTHF)/THE ratio (1.02 [0.84-1.27] vs 0.94 [0.79-1.0], P<0.001) and cortisol/cortisone ratio (0.70 [0.58-0.83] vs 0.63 [0.54-0.74], P<0.001) than healthy controls. In T2D, lower eGFR was associated with a higher (THF+aTHF)/THE ratio (β=-0.35, P<0.001), and a higher cortisol/cortisone ratio (β=-0.16, P=0.001).

**Conclusions**
In this real life secondary care setting of patients with T2D, 11β-HSD enzymes activities were shifted to higher intracellular cortisol production in T2D, which was further aggravated in patients with CKD. Prospective analyses are warranted to investigate causality of these associations.

# Introduction

Metabolic similarities between patients with type 2 diabetes (T2D) and patients with hypercortisolism (Cushing's syndrome or glucocorticoid treatment) have given rise to the hypothesis that relative hypercortisolism might occur in T2D[1]. Although overt hypercortisolism is not typical in T2D, intracellular cortisol exposure is potentially increased through upregulation of 11-beta hydroxysteroid dehydrogenase type 1 (11β-HSD1), which regenerates inactive cortisone to active cortisol in the liver and in adipose tissue, or downregulation of 11-beta hydroxysteroid dehydrogenase type 2 (11β-HSD2), which reduces active cortisol to inactive cortisone (figure 1). Sporadic studies have found signs that 11β-HSD activities are shifted towards higher intracellular cortisol production in T2D, compared to non-T2D subjects[2-4], although results are conflicting[5]. In patients with chronic kidney disease, similar shifts in 11β-HSD activities have been suggested[6,7]. However, 11β-HSD activities in patients with T2D with renal function impairment have never been formally investigated.

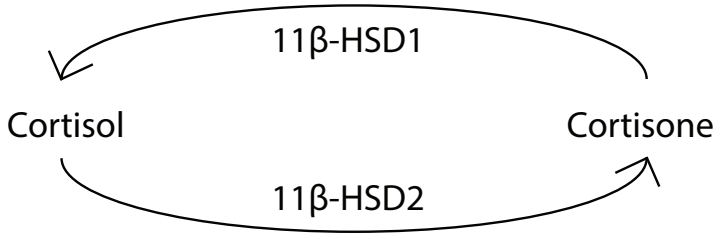


**Figure 1**. Effect of 11β-HSD activites on intracellular cortisol levels. 11β-HSD1 increases cortisol levels by regenerating inactive cortisone to active cortisol. 11β-HSD2 decreases cortisol levels by reducing active cortisol to inactive cortisone.

Interest in 11β-HSD pathways has recently been refueled after the development of several compounds which inhibit 11β-HSD1. Phase II preclinical trials with such agents have shown improved glycemic control, lipid profile and blood pressure, and even demonstrated modest weight loss[8]. However, effects on each separate component of the metabolic syndrome were relatively small. If 11β-HSD1 activity is highest in T2D with renal function impairment, this could mean that pharmacological 11β-HSD1 inhibition could be a promising treatment option specifically for these patients.

In the present study, we therefore quantified total urinary excretion of cortisol, cortisone and their metabolites (tetrahydrocortisol, THF; allo-THF, aTHF; tetrahydrocortisone, THE) with the aim of estimating (1) whether 11β-HSD activities differ between patients with T2D and healthy controls, and (2) to investigate whether there is an association between eGFR and 11β-HSD activities in patients with T2D.

# Methods

We performed a cross-sectional analysis in baseline data from the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1). The study design was described in detail elsewhere[9]. The study was approved by the local institutional review board (METC-Twente, registration number: NL57219.044.16) and the institutional review board in the University Medical Centre Groningen (METC-Groningen registration number: 1009.68020), and is registered in the Netherlands Trial Register (NTR trial code 5855). The study was performed according to the guidelines of good clinical practice and the declaration of Helsinki.

*Participants*

All patients with T2D treated in the outpatient clinic of our hospital, aged 18+ years, were eligible for the study. Exclusion criteria were inability to understand the informed consent procedure, insufficient command of the Dutch language, or dialysis dependency. As a control group reflecting the general population, we included 275 healthy subjects who participated in a screening program before kidney donation in the University Medical Centre Groningen. None of the healthy controls had a history of diabetes, cardiovascular events or kidney disease. Hypertension, if present, was treated with a maximum of one class of antihypertensive drugs.

*Study procedures*

Eligible patients were selected from the electronic patient file. At the clinic, sociodemographic characteristics, medical history, lifestyle behaviors, and current medications were recorded. Height, weight, waist and hip circumference were measured. Body mass index was calculated as weight divided by height squared ($kg/m^2$), and body surface area was estimated by applying the universally adopted formula of DuBois. Blood pressure was measured in a supine position by an automated device (Dinamap®; GE Medical systems, Milwaukee, WI) for 15 minutes with a one-minute interval. The mean systolic and diastolic pressure of the final three measurements was used for further analysis.

Blood was drawn from venipuncture for routine laboratory measurements. From a 24-hour urine collection the following parameters are measured: sodium, potassium, creatinine, calcium, phosphate and uric acid excretion. For the proper collection of the 24h urine sample, patients were instructed to dispose the first morning void urine, and thereafter collect all urine in the provided canister until the first morning void urine of the next day. In between voids, they were instructed to store the canister in a dark cool place, preferably in a refrigerator. Samples of blood and 24h urine were stored for later analysis.

*Cortisol measurements*

Urinary cortisol, cortisone, THF, aTHF, and THE concentrations in 24h urine samples were measured using a validated high-performance liquid chromatography tandem mass spectrometry (LC-MS/MS) assay as previously described[10]. For all components, stable isotope labeled internal standards were added and the mixtures were incubated with an enzyme solution consisting of sulfatases and β-glucuronidases (Suc d'Helix Pomatia, Pall Biopharmaceuticals, Port Washington, NY), to ensure hydrolysis of cortisol and the metabolites from their sulfated and glucuronidated forms. In contrast to the more generally applied urinary free cortisol measurement, this method measures total cortisol and its metabolites. Subsequently, the analytes were extracted using a Supported Liquid Extraction technique. Finally, separation and detection were performed by use of a CSH Phenyl-Hexyl column (particle size 1.7 µm, 2.1 mm internal diameter by 100 mm; Waters) and a XEVO TQ-s® tandem mass spectrometer operated in negative electrospray ionization mode (Waters, Milford, MA), respectively. Intra- and inter-assay variation coefficients were <5.7% and <9.8%, respectively. Prednisone and prednisolone were chromatographically separated from cortisol and its metabolites and therefore did not interfere. Total 24h urinary excretions were calculated by multiplying the concentrations by 24h urinary volume.

The urinary ratios of (THF+aTHF)/THE and cortisol/cortisone are widely used to assess enzyme activity of 11β-HSD1 and 11β-HSD2. The urinary cortisol/cortisone ratio is considered to reflect activity of 11β-HSD2, while urinary (THF+alloTHF)/THE ratio is considered as an overall measure of whole body 11β-HSD activity[11-13].

*Data analysis and statistics*

Statistical analyses were performed using Statistical Package for the Social Sciences (IBM, Chicago, IL, USA), version 22.0. Normality of data was assessed by visually inspecting the frequency histograms. Normally distributed data is shown as mean ± standard deviation, skewed data is shown as median [interquartile range], and nominal data as number of patients (percentage). Differences between patients with T2D and healthy controls were tested using linear regression analyses, unadjusted and while adjusting for potential confounders such as gender, age and Body Mass Index (BMI).

Using R software, the univariate associations between estimated Glomerular Filtration Rate (eGFR) and the (THF+aTHF)/THE and cortisol/cortisone ratios were assessed with generalized additive models (mgcv package; The R-Foundation for Statistical Computing, Vienna, Austria) as described previously[14]. The model effect and nonlinearity were tested with the use of 2-sided Wald tests. P-nonlinearity values were calculated by comparing restricted cubic spline terms to linear models.

To identify possible confounders, we determined the associations between clinical parameters and the (THF+aTHF)/THE and cortisol/cortisone ratios using linear regression analyses. Followingly, we performed multivariate linear regression to determine the association between eGFR and the (THF+aTHF)/THE and cortisol/cortisone ratios, while adjusting for common confounders and for parameters with a $P<0.15$ in univariate analysis. In order to test for gender differences we also performed the analyses for men and women separately.

# Results

Data on urinary cortisol excretion were available in 373 patients of DIALECT-1 and in 275 healthy controls (Table 1). In T2D patients, the mean age was 64 ± 9 years, the majority were men (58%), and mean BMI was 32.8 ± 6.0 kg/m$^2$. The majority of T2D patients had one or more microvascular complications (70%), with nephropathy being the most prevalent (49%), and macrovascular complications were present in 39% of patients. In the healthy controls, there were fewer men (48%; P=0.008), participants were younger (53 ± 11; P<0.001), had a lower BMI (25.9 ± 3.5; P<0.001) and a higher eGFR (91 ± 24 vs 78 ± 24 T2D; P<0.001), as compared to T2D patients.

**Table 1**. Patients characteristics and urinary excretion of cortisol metabolites

| | *Healthy controls* | *Type 2 Diabetes* | *Standardized* β | | |
|---|---|---|---|---|---|
| *Patient characteristics* | *n=275* | *n=373* | *Model 1* | *Model 2* | *Model 3* |
| Men, n (%) | 132 (48) | 215 (58) | -0.10** | | |
| Age, years | 53 ± 11 | 64 ± 9 | 0.43*** | | |
| BMI, kg/m$^2$ | 25.9 ± 3.5 | 32.8 ± 6.0 | 0.53*** | 0.58*** | |
| Systolic blood pressure, mmHg | 125 ± 14 | 136 ± 16 | 0.33*** | 0.26*** | 0.22*** |
| Diastolic blood pressure, mmHg | 76 ± 9 | 74 ± 10 | 0.06 | -0.02 | 0.002 |
| HbA1c, mmol/mol | 38 ± 4 | 57 ± 12 | 0.71*** | 0.70*** | 0.70*** |
| eGFR, ml/min•1.73m$^2$ | 91 ± 14 | 78 ± 24 | -0.29*** | -0.05 | -0.009 |
| eGFR<60 ml/min•1.73m$^2$, n (%) | 4 (1%) | 90 (20) | | | |
| eGFR<30 ml/min•1.73m$^2$, n (%) | 0 (0) | 14 (3) | | | |
| Increased albuminuria, n (%) | 15 (6) | 136 (31) | 0.30*** | 0.25*** | 0.24*** |
| Microvascular disease, n (%) | *n.a.* | 260 (70) | | | |
| Macrovascular disease, n (%) | *n.a.* | 144 (39) | | | |
| ***Urinary excretion of cortisol metabolites*** | | | | | |
| Urinary cortisol excretion, nmol/24h | 332 [244-445] | 274 [204-400] | -0.15*** | -0.15** | -0.16** |
| Urinary cortisone excretion, nmol/24h | 526 [418-648] | 408 [308-549] | -0.25*** | -0.24*** | -0.24*** |
| Urinary THF excretion, μmol/24h | 6.9 [5.1-9.3] | 7.0 [5.3-9.5] | 0.02 | -0.005 | -0.11* |
| Urinary LN a-THF excretion, μmol/24h | 4.2 [2.6-6.5] | 4.8 [2.7-7.5] | 0.07 | 0.05 | -0.07 |
| Urinary THE excretion, μmol/24h | 12.5 [8.5-16.8] | 11.5 [8.1-15.3] | -0.06 | -0.03 | -0.16** |
| Summated urinary cortisol and metabolites excretion, μmol/24h | 24.6 [17.4-33.7] | 24.3 [18.5-32.3] | -0.02 | -0.01 | -0.15** |
| (THF+aTHF)/THE, μmol/μmol | 0.94 [0.79-1.0] | 1.02 [0.84-1.27] | 0.19*** | 0.12** | 0.11* |
| Cortisol/cortisone, nmol/nmol | 0.63 [0.54-0.74] | 0.70 [0.58-0.83] | 0.15*** | 0.14** | 0.14* |

Differences between groups were tested via univariable and multivariable linear regression analyses of which standardized βs are presented (*P<0.05, **P<0.01, ***P<0.001). Model 1 is a crude model. Model 2 was adjusted for age and gender. Model 3 was adjusted as for model 2 and for BMI. BMI, body mass index; BSA, body surface area; eGFR, estimated glomerular filtration rate; THF, tetrahydrocortisol; aTHF, alloTHF; THE, tetrahydrocortisone.

*Total 24h urinary excretion of cortisol and cortisol metabolites in T2D patients and healthy controls*
Urinary excretion of cortisol and its metabolites in patients with T2D and healthy controls is demonstrated in Table 1. The median urinary excretion of cortisol and cortisone was lower in T2D than in healthy controls (cortisol 274 [204-400] vs 332 [244-445] nmol/24h; P<0.001; cortisone 408 [308-549] vs 526 [418-648] nmol/24h; P<0.001). However, the ratio of cortisol/cortisone was higher in patients with T2D (1.02 [0.84-1.27] vs 0.94 [0.79-1.00], P<0.001), also when adjusting for age, gender and BMI. In addition, there was no difference in urinary excretion of THF, aTHF, THE and summated cortisol and metabolites between patients with T2D and healthy controls. The (THF+aTHF)/THE ratio, however, was again higher in T2D (0.70 [0.58-0.83] vs 0.63 [0.54-0.74]; P<0.001), also after adjustment for confounders. Differences in both ratios between patients with T2D and healthy controls were similar in men and women (data not shown).

*Associations between eGFR and the (THF+aTHF)/THE ratio and cortisol/cortisone ratios in T2D.*
In patients with T2D, there was an inverse linear association between eGFR and the log transformed (THF+aTHF)/THE ratio (β=-0.35, P<0.001, P-nonlinearity=0.14; figure 2A). In addition, eGFR was also inversely associated with the cortisol/cortisone ratio (β=-0.16, P=0.001, P-nonlinearity=0.27, figure 2B).

As secondary analyses, we also investigated the associations of the (THF+aTHF)/THE and the cortisol/cortisone ratios with clinical characteristics (Table 2). We found that the (THF+aTHF)/THE ratio was associated with gender (β=-0.14, P=0.006), age (β=0.16, P=0.002), systolic blood pressure (β=-0.13, P=0.01), diastolic blood pressure (β=-0.14, P=0.008), presence of coronary heart disease (β= 0.16, P=0.002), beta blocker use (β=0.14, P=0.005), loop diuretic use (β=0.13, P=0.01) and plasma LDL cholesterol (β=-0.15, P=0.006). Insulin use was not significantly associated with the (THF+aTHF)/THE ratio, and in insulin users there was no association between cumulative daily insulin dosage and the (THF+aTHF)/THE ratio. Of note, adjustment for factors associated with the (THF+aTHF)/THE ratio did not markedly influence the association between eGFR and (THF+aTHF)/THE (fully adjusted model: β=-0.37, P<0.001; Table 3). The association between eGFR and the (THF+aTHF)/THE ratio was similar for men and women (data not shown).

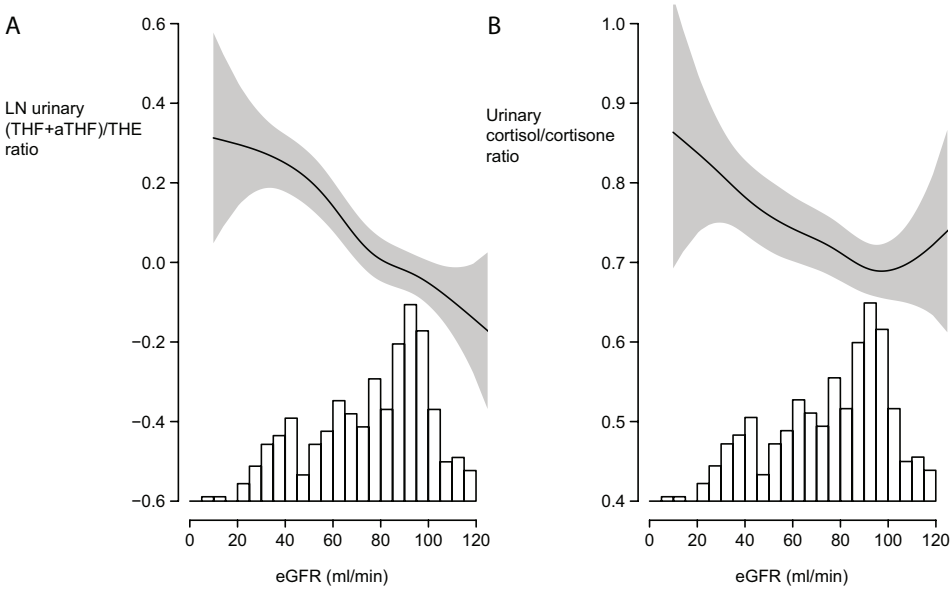


**Figure 2.** Continuous associations of the eGFR with A) the log transformed urinary (THF+aTHF)/THE and B) the cortisol/cortisone ratios in patients with T2D (*n*=373). Continuous associations were modeled via generalized additive models. Shaded areas represent the corresponding 95% CIs. The histograms illustrate distributions of plasma eGFR in T2D patients. For the association between eGFR and LN (THF+aTHF)/THE, P-nonlinearity was 0.14, and the β was -0.35 (P<0.001). In case of the urinary cortisol/cortisone ratio, the P-nonlinearity was 0.27, and the β was -0.16 (P=0.001).

In parallel, the cortisol/cortisone ratio was associated with the presence of cerebrovascular disease (β=0.17, P=0.001) and loop diuretic use (β=0.10, P=0.05). Plasma aldosterone concentration and 24h urinary aldosterone excretion were not associated with the cortisol/cortisone ratio. Again, the association between eGFR and the cortisol/cortisone ratio was unaltered by adjustment for possible confounders (fully adjusted model: β=-0.14, P=0.03; Table 4). Additionally, the β of the association between eGFR and the cortisol/cortisone ratio was similar in men and women, although the association only statistically significant in men (fully adjusted models: β=-0.17, P=0.05 for men, β=-0.15, P=0.14 for women).

**Table 2**. Unadjusted associations between clinical parameters and the (THF + aTHF)/THE and cortisol/cortisone ratios in patients with type 2 diabetes

| *n=374* | *LN (THF + aTHF)/THE* | | *Cortisol/Cortisone* | |
|---|---|---|---|---|
| *Patient characteristics* | *Stand* β | *P-value* | *Stand* β | *P-value* |
| Women | -0.14 | 0.006 | -0.01 | 0.83 |
| Age, years | 0.16 | 0.002 | 0.09 | 0.08 |
| BMI, kg/m² | <0.01 | 0.98 | 0.04 | 0.43 |
| Current smoker | -0.02 | 0.66 | 0.10 | 0.06 |
| Alcohol use (yes/no) | -0.02 | 0.73 | -0.02 | 0.70 |
| Systolic blood pressure, mmHg | -0.13 | 0.01 | 0.03 | 0.62 |
| Diastolic blood pressure, mmHg | -0.14 | 0.008 | 0.00 | 0.95 |
| Heart frequency, beats/min | -0.08 | 0.13 | 0.10 | 0.06 |
| *Co-morbidity* | | | | |
| Microvascular disease | 0.17 | 0.001 | 0.10 | 0.05 |
| Retinopathy | 0.03 | 0.58 | 0.02 | 0.74 |
| Neuropathy | 0.05 | 0.39 | 0.01 | 0.79 |
| Nephropathy | 0.12 | 0.02 | 0.10 | 0.07 |
| Macrovascular disease | 0.17 | 0.001 | 0.08 | 0.11 |
| Coronary heart disease | 0.16 | 0.002 | 0.00 | 0.96 |
| Cerebrovascular disease | 0.10 | 0.06 | 0.17 | 0.001 |
| Peripheral artery disease | 0.06 | 0.25 | 0.02 | 0.73 |
| *Pharmacological treatment* | | | | |
| RAAS inhibition | <0.001 | 0.99 | -0.02 | 0.72 |
| Bèta-blocker | 0.14 | 0.005 | 0.02 | 0.67 |
| Calcium antagonist | 0.05 | 0.30 | 0.02 | 0.69 |
| Thiazide diuretics | 0.02 | 0.73 | -0.05 | 0.36 |
| Loop diuretics | 0.13 | 0.01 | 0.10 | 0.05 |
| Potassium saving diuretics | 0.11 | 0.04 | 0.02 | 0.78 |
| Insulin | 0.03 | 0.51 | -0.08 | 0.15 |
| Cumulative insulin dosage, units/day | -0.09 | 0.21 | -0.002 | 0.98 |
| *Serum values* | | | | |
| Serum HbA1c, mmol/mol | -0.10 | 0.06 | 0.05 | 0.36 |
| Plasma total cholesterol, mmol/l | -0.15 | 0.004 | -0.02 | 0.75 |
| Plasma LDL-cholesterol, mmol/l | -0.15 | 0.006 | 0.04 | 0.48 |
| Plasma HDL-cholesterol | -0.06 | 0.24 | -0.07 | 0.20 |
| Plasma aldosterone concentration, pg/ml | 0.08 | 0.17 | 0.07 | 0.22 |
| *Urinary excretion* | | | | |
| LN Urinary albumin excretion, mg/24h | 0.07 | 0.22 | 0.05 | 0.39 |
| Urinary Cortisol/cortison, nmol/nmol | 0.20 | <0.001 | | |
| LN (THF+aTHF)/THE, µmol/µmol | | | 0.20 | <0.001 |
| Urinary aldosterone excretion, µg/24h | 0.02 | 0.72 | -0.04 | 0.45 |

Associations were tested using univariate linear regression of which standardized βs and P-values are presented. LN, natural logarithm; BMI, body mass index; BSA, body surface area; eGFR, estimated glomerular filtration rate; THF, tetrahydrocortisol; aTHF, alloTHF; THE, tetrahydrocortisone.

**Table 3**. Association between eGFR and the urinary (THF+aTHF)/THE ratio in patients with type 2 diabetes after adjustments for possible confounders

| | | LN (THF + aTHF)/THE | |
|---|---|---|---|
| | | *Stand* β | *P-value* |
| Model 1 | eGFR, ml/min•1.73m² | -0.36 | <0.001 |
| Model 2 | eGFR, ml/min•1.73m² | -0.39 | <0.001 |
| Model 3 | eGFR, ml/min•1.73m² | -0.38 | <0.001 |
| Model 4 | eGFR, ml/min•1.73m² | -0.38 | <0.001 |
| Model 5 | eGFR, ml/min•1.73m² | -0.37 | <0.001 |
| Model 6 | eGFR, ml/min•1.73m² | -0.37 | <0.001 |

Associations were tested using multivariate linear regression of which standardized βs and P-values are presented.
Model 1 is a crude model.
Model 2 was adjusted for age and gender.
Model 3 was adjusted for Model 2 + coronary artery disease (no/yes), cerebrovascular disease (no/yes).
Model 4 was adjusted for Model 3 + BMI (kg/m²), alcohol intake (none/any), current smoking (no/yes).
Model 5 was adjusted for Model 4 + systolic blood pressure (mmHg), diastolic blood pressure (mmHg), heart frequency (beats/min), LDL cholesterol (mmol/l).
Model 6 was adjusted for Model 5 + beta blocker use (no/yes), loop diuretic use (no/yes), potassium saving diuretic use (no/yes). LN, natural logarithm; eGFR, estimated glomerular filtration rate; THF, tetrahydrocortisol; aTHF, alloTHF; THE, tetrahydrocortisone.

**Table 4**. Association between eGFR and the urinary cortisol/cortisone ratio in patients with type 2 diabetes after adjustments for possible confounders

| | | Cortisol/cortisone | |
|---|---|---|---|
| | | Stand β | P-value |
| Model 1 | eGFR, ml/min•1.73m² | -0.17 | 0.001 |
| Model 2 | eGFR, ml/min•1.73m² | -0.17 | 0.007 |
| Model 3 | eGFR, ml/min•1.73m² | -0.14 | 0.02 |
| Model 4 | eGFR, ml/min•1.73m² | -0.14 | 0.03 |
| Model 5 | eGFR, ml/min•1.73m² | -0.16 | 0.01 |
| Model 6 | eGFR, ml/min•1.73m² | -0.14 | 0.03 |

Associations were tested using multivariate linear regression of which standardized βs and P-values are presented. Model 1 is a crude model. Model 2 was adjusted for age and gender. Model 3 was adjusted for Model 2 + cerebrovascular disease (no/yes). Model 4 was adjusted for Model 3 + BMI (kg/m²), alcohol intake (none/any), current smoking (no/yes). Model 5 was adjusted for Model 4 + heart frequency (beats/min). Model 6 was adjusted for Model 5 + loop diuretic use (no/yes), insulin use (no/yes). eGFR, estimated glomerular filtration rate

# Discussion

To our knowledge this is the first study on 11β-HSD activities in patients with T2D with a large subgroup of patients with renal function impairment. We found that both the urinary (THF+aTHF)/THE and cortisol/cortisone ratios were higher in patients with T2D as compared to healthy controls. Additionally, lower renal function was associated with higher urinary (THF+aTHF)/THE and cortisol/cortisone ratios in patients with T2D. These findings suggest that in T2D, intracellular cortisol exposure is increased, and that in diabetic kidney disease, there is even further derangement of 11β-HSD activity towards intracellular cortisol production.

We measured urinary excretion of cortisol and its metabolites using LC-MS/MS, after hydrolysis from their sulfated and glucuronidated forms. Because in other literature the hydrolysis step usually is not performed, we report a higher excretion of cortisol and its metabolites, and therefore direct comparison of our data with previous literature is not possible.

In line with our findings, prior observations in small groups of patients with T2D reported a shift towards higher intracellular cortisol production in patients with T2D as compared to healthy controls[2,3]. Studies assessing solely 11β-HSD1 activity, by measuring the conversion of labeled cortisol to cortisone, reported higher activity of 11β-HSD1 in patients with T2D than in overweight/obese controls without T2D[2,3]. On the other hand, Valsamakis found no statistically significant difference in the urinary (THF+aTHF)/THE and cortisol/cortisone ratios between patients with T2D and controls[5]. It should be noted that in the latter study, patients were in an earlier disease stage than in our study; patients were younger, there were no insulin users and patients with renal function impairment were excluded, while in our study, the median diabetes duration was 11 [7-18] years, and approximately two-thirds of patients used insulin. Lavery *et al*. previously reported an increased frequency of short alleles of the 11β-HSD2 gene in T1D, suggesting reduced activity of 11β-HSD2 in diabetes, however without measurement of in-vivo 11β-HSD2 activity[4]. Our larger study adds to these findings by illustrating that in established T2D in a real-life setting, both total body 11β-HSD activity and 11β-HSD2 activity are shifted towards higher intracellular cortisol exposure in T2D, independent of age, gender and BMI.

Although the mechanisms behind derangement of 11β-HSD activities in T2D is unknown, Anderson *et al*. previously described that metformin may increase whole body 11β-HSD1 activity, potentially diminishing other metabolic effects of metformin[15]. Here we found a non-statistically significant trend towards lower 11β-HSD1 activity in patients

on metformin. However, it should be noted our population represents patients with long-standing T2D (11 [7-18] years), complicating direct comparison of results. Prospective research is needed to further clarify the effects of metformin on 11β-HSD1 activity.

In patients with T2D, we found that the urinary (THF+aTHF)/THE and cortisol/cortisone ratios are inversely associated with eGFR. To our knowledge, the association between 11β-HSD activity and eGFR in T2D has not been reported previously. Our finding is supported by a previous study by Quinkler *et al*., which demonstrated an inverse association between creatinine clearance and the urinary (THF+aTHF)/THE and cortisol/cortisone ratios in non-diabetic patients with renal function impairment[6]. In line, Whitworth *et al*. reported an association between higher plasma creatinine and lower plasma cortisone levels in non-diabetic CKD patients, indicative of an association between lower eGFR and lower 11β-HSD2 activity[16]. The mechanism underlying the association between lower renal function and altered 11β-HSD activities is unknown. In (diabetic) chronic kidney disease, there is neuroendocrinological derangement, with increased sympathetic activity, illustrated by higher inflammatory markers, and higher cortisol and aldosterone levels in CKD[17-20]. Possibly, in chronic kidney disease, alteration of 11β-HSD activities is part of this neuroendocrinological derangement. Indeed we found that a trend between higher C-reactive protein and altered 11β-HSD activities, which is in line with previous research on 11β-HSD activities in inflamed tissues[21,22]. However, as of now it is unknown whether a higher degree of inflammation leads to 11β-HSD dysregulation or vice versa. In case of the latter, altered 11β-HSD activities could be associated with inflammation-related complications commonly seen in CKD, such as insulin resistance, dyslipidemia and hypertension[23-25]. Additionally, altered cortisol handling may have consequences for clinical outcomes in CKD, previously, Himmelfarb *et al*. demonstrated that higher predialysis serum cortisol levels were associated with higher rates of hospitalization and malnutrition[26]. It should be noted that in our cohort there were few patients with end-stage renal failure (eGFR <15 ml/min•1.73m2). It would be interesting to investigate whether the association between eGFR and 11β-HSD activities remains linear if the population would be expanded with patients with more severe impairment of renal function and patients approaching end-stage renal failure.

Moreover, 11β-HSD2 inactivates cortisol in the intracellular space, thus avoiding cortisol mediated mineralocorticoid receptor (MR) activation. Lower 11β-HSD2 activity in those with renal function impairment suggests increased MR activation by cortisol. MR activation has been associated with a plethora of detrimental effects on target organs, such as the kidneys, the heart and the vasculature, which can be blocked by MR antagonism[27-34]. Therefore, lower 11β-HSD2 activity in T2D could play an important role in the development and course of diabetic nephropathy. However, it should be noted that in the

present study we found no association between 11β-HSD2 and markers of MR activation such as plasma aldosterone concentration, urinary aldosterone excretion, blood pressure and hypokalemia, although such relations might well be disturbed by unstandardized sodium intake and frequent use of antihypertensives interfering in the renin-angiotensin-aldosterone-system.

Our study has several strengths. First, this is the largest study to date on 11β-HSD activities in patients with T2D and renal function impairment. Because previous assays for measuring cortisol metabolites were difficult to perform in a large group of patients, little data is available on the epidemiology of 11β-HSD activities. Second, the broad inclusion criteria of DIALECT allow us to study a group of real-world patients with T2D treated in secondary care, with minimal inclusion bias. The primary limitation of the study is the cross-sectional design, which does not allow conclusions on causality. Therefore, the results of our study should be seen as predominantly hypothesis generating. In-depth prospective studies are necessary to validate our findings. Furthermore, it should be noted the urinary (THF+aTHF)/THE ratio is an indirect marker of whole body 11β-HSD enzyme activity, and therefore should be interpreted with caution, because 11β-HSD activity might differ between different tissues. Additionally, the (THF+aTHF)/THE ratio can only be used to interpret 11β-HSD1 activity if the urinary cortisol/cortisone ratios is unaltered. Therefore, additional studies are necessary to assess whether in T2D and CKD 11β-HSD1 activity specifically is altered similarly as 11β-HSD2 activity. Also, due to the fact that blood was taken in a non-fasting state, data on fasting glucose and homeostatic model assessment for insulin resistance were unavailable in DIALECT. Therefore the association between 11β-HSD activities and insulin resistance could not be assessed directly. Lastly, previously it has been demonstrated that higher urinary cortisol excretion is associated with oxidative stress[35]. It would be interesting to investigate whether the 11β-HSD derangement in T2D and CKD we report here is also associated with markers of oxidative damage, however data on markers of oxidative stress were not available in this study.
Our findings have several potential clinical implications. The shift towards higher intracellular cortisol production by 11β-HSD enzymes in T2D with renal function impairment could indicate that 11β-HSD1 inhibitors might have an increased beneficial effect in patients with diabetic nephropathy. Additionally, the lower 11β-HSD2 activity in T2D and renal function impairment might have implications for treatment with MR antagonists, especially in diabetic nephropathy. Future prospective studies are necessary to validate our findings, and assess the association between 11β-HSDs derangements and clinical adverse outcomes. Additionally, future studies should be performed to evaluate whether alterations between 11β-HSD in T2D patients with CKD are similar to CKD-patients without T2D.

## Conclusions

This is the largest study to date on 11β-HSD activities in a real life secondary care setting of patients with T2D. We found that activity of 11β-HSDs is shifted towards higher intracellular cortisol production in T2D, and especially in those with T2D and renal function impairment. This could have important implications for the use of both 11β-HSD1 inhibitors and MR antagonists in T2D and renal function impairment.

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# CHAPTER 11

## Summary
and general discussion

# Interlude

Cardiovascular disease (CVD) is one of the most important causes of morbidity and mortality worldwide, and therefore CVD prevention is a major focus of healthcare[1-3]. It has been estimated that 80% of CVD can be prevented by adequate intervention in modifiable risk factors[4]. However, in clinical practice such a risk reduction is rarely achieved, in part or in whole, because targets on blood pressure, LDL-cholesterol and HbA1c are not reached in a majority of patients. Studies on why these targets are not reached mostly focus on either pharmacological treatment or lifestyle treatment. However, an integrated approach of the combined roles of pharmacology and lifestyle in CVD risk reduction is warranted, because both are part of treatment. Therefore, we initiated the DIAbetes and Lifestyle Cohort Twente (DIALECT), a prospective cohort study in which we gathered extensive data on clinical condition, pharmacological treatment and lifestyle habits in a high-risk population of type 2 diabetes mellitus (T2DM) patients, treated in routine secondary health care. In **part 1** of this thesis we performed an integrated assessment of lifestyle and pharmacological intervention on achievement of blood pressure (**Chapter 2**), LDL cholesterol (**Chapter 3**) and HbA1c (**Chapter 4**) targets. Additionally, as magnesium deficiency is an emerging risk factor for CVD, and is associated with poor dietary quality[5], we studied the association between magnesium parameters (intake, 24h urinary excretion and plasma concentration), and coronary heart disease (**Chapter 5**). Lastly, we compared results from an objective and subjective assessment of weekly physical activity (**Chapter 6**).

Next, to improve treatment of established risk factors, it is important to unravel pathophysiological mechanisms behind increased risk, so that pharmacological treatment in CVD prevention can be further optimized. In **part 2** of the thesis we investigated neurohumoral activation in diabetes and chronic kidney disease (CKD), two clinical conditions that are associated with highly increased CVD risk. We studied available literature on non-classical effects of aldosterone (**Chapter 7**). Additionally, we studied the regulation and effects of aldosterone in healthy controls (**Chapter 8**), and chronic kidney disease patients (**Chapter 9**). Lastly, we studied intracellular cortisol production in patients with T2DM and renal function impairment (**Chapter 10**).

# Summary

## Part 1. Integrated assessment of pharmacological and lifestyle treatment in routine cardiovascular risk management of patients with T2DM in secondary care.

### Blood pressure treatment in T2DM: Opportunities for pharmacological and lifestyle treatment.

Hypertension is one of the most important CVD risk factors and reducing blood pressure (BP) is associated with a substantial risk reduction of future CVD. BP reduction is especially important in T2DM, in which the prevalence of hypertension has been estimated to be 70-80%[6]. However, in clinical practice a large number of patients does not reach the target blood pressure, and it is unclear how BP target achievement can be improved. Therefore, in **Chapter 2** we studied 1) to which extent blood pressure targets were reached in high-risk patients with T2DM in routine secondary health care, and 2) pharmacological and nutritional management in patients on target and not on target BP in the DIAbetes and LifEstyle Cohort Twente-1 (DIALECT-1; *n*=450). In DIALECT extensive data is gathered on patient characteristics, clinical status, biochemical characteristics, pharmacological treatment, dietary habits and physical activity. Blood pressure was measured at baseline during 15 minutes, with one-minute intervals. Nutritional intake of sodium, potassium, phosphate and magnesium was determined from their 24h urinary excretion.

In this population, only half of the patients had blood pressure on target, despite the fact that the majority was on antihypertensive medication (81%). In other high-risk T2DM populations, similar percentages of BP target achievement were reported[7-9]. In primary care, 85% of patients with T2DM reached target BP, with similar antihypertensive as in our population[10,11]. It must be stated, however, that the diabetes population in primary care in the Netherlands is diluted with a considerable proportion of very mild diabetes cases, i.e. patients with mildly elevated blood glucose reaching HbA1c <53 mmol/mol with only 1-2 tablets of metformin per day. Our findings illustrate a more complex patient population in secondary care as compared to primary care.

Study of both pharmacological and nutritional factors in patients with BP not on target allowed us to identify the opportunities for improving BP target achievement. Regarding pharmacological treatment, two thirds of the patients with BP not on target used 0-2 antihypertensives, therefore in those patients there is opportunity to increase pharmacological treatment. Because renin-angiotensin-aldosterone system inhibition (RAASi) and beta-blockers were the most frequently used antihypertensive drugs, reduction of extracellular volume with thiazide diuretics, or dietary sodium restriction (see below), could be

a good next step. In contrast, about one third of the patients with BP not on target already received three or more antihypertensive agents, indicating treatment resistant hypertension, as defined in guidelines as ongoing hypertension despite treatment with three maximally dosed antihypertensives, of which at least one is a diuretic. Although options to further increase pharmacological treatment are limited in these patients, mineralocorticoid receptor antagonists (MRA) could be a good choice[12]. The PATHWAY-2 trial has illustrated that spironolactone is more effective in reducing BP in resistant hypertension than bisoprolol or doxazosin[13]. Although adding MRA to antihypertensive regimens is mostly safe, in combination with another inhibitor of the renin-angiotensin-aldosterone system monitoring should be performed for hyperkalaemia, and in combination with another diuretic, for acute renal failure[12].

Regarding lifestyle intervention there were ample opportunities for improvement, of which weight loss and reducing dietary salt intake were the most prominent. It should be noted that lifestyle guideline adherence was equally poor in the BP on target and BP not on target groups, and therefore intervention to improve lifestyle is advisable in all patients. Lifestyle intervention is not only beneficial for BP reduction, but also reduces CVD risk, improves glycaemic control and reduces LDL cholesterol[14-18]. However, in those with BP not on target, there is more urgency to change lifestyle to achieve the target BP. First, obesity was highly prevalent: the mean BMI was 32.9 ± 6.2 kg/m$^2$ and 65% of patients had a BMI >30 kg/m$^2$. Weight loss has been well-established to reduce blood pressure; even a weight reduction of 5 kg can reduce both systolic and diastolic BP by 4 mmHg[19]. Moreover, weight loss can reduce the number of needed antihypertensive agents, also in non-obese subjects[20]. Second, dietary salt intake was high, 11 ± 5 g/day, with only 12% of the population consuming ≤6 g/day. This was considerably higher than the salt intake in the general Dutch population of 9 g/day[21]. Reducing salt intake is a compelling option in this population, as even a modest reduction in salt intake (from 12 to 9 g/day) can reduce BP by 6/3 mmHg[22]. Moreover, reducing salt intake can potentiate the antihypertensive effects of RAASi, in addition to the BP lowering effects of reducing extracellular volume[23-25].

Our observations provide insight into real-life cardiovascular risk management in high-risk patients with T2DM. The abundance of antihypertensive drug prescriptions, and the low frequency of lifestyle guideline adherence, illustrates that the emphasis in this population lies on pharmacological treatment. However, hypertension is a progressive condition, with increasing BP as patients get older[26]. Therefore, in the course of T2DM, a significant number of patients develops resistant hypertension, and pharmacological treatment alone does not suffice to reach the target BP[26]. Adding lifestyle intervention to pharmacological treatment can reduce BP and the number of needed pharmacological

agents, and therefore delay progression into true resistant hypertension, even in already advanced disease. Therefore, although lifestyle intervention is notoriously difficult to achieve, it should not be overlooked.

Taken together, in patients with BP not on target, in roughly two thirds of the patients there is still room to increase pharmacological treatment, and in roughly one third options are limited due to treatment resistant hypertension. On the other hand, in almost all patients there is ample opportunity to reduce BP through lifestyle measure, most notably by weight reduction and reduction of dietary sodium intake.

### Opportunities in LDL cholesterol treatment in T2DM: statins are the most important factor associated with target achievement.

In addition to high blood pressure, high LDL cholesterol (LDLc) is a major risk factor for CVD morbidity and mortality. LDLc reduction with statin therapy in T2DM is associated with reduction of CVD events, both in primary and secondary prevention[27-30]. Despite the beneficial effects of LDLc lowering, a large proportion of patients with T2DM (44-67%) does not achieve the recommended treatment targets[31-33]. Therefore, in **Chapter 3** we studied the proportion of patients that achieved the LDLc treatment target in DIALECT-1 and studied opportunities to improve pharmacological and lifestyle LDLc management.

We found that three quarters of patients were on statin treatment, and that the target LDLc was reached in roughly three quarters of patients, which is somewhat higher than reported in literature[31-33]. Patients with LDLc of 2.5 mmol/l or lower more often used statins, and intensity of statin treatment was higher. In patients with LDLc >2.5 mmol/l, more than half of patients did not use statins, and only a very small group (8%) used high intensity statin treatment. In contrast to blood pressure treatment, where treatment resistance was found in roughly a third of cases (**Chapter 2**), our data suggest that treatment resistance to statins is rare. Therefore, we propose that the first step in improving LDLc target achievement in secondary care should be initiation or intensification of statin treatment.

In clinical practice, statin treatment is often discontinued due to muscle related side-effects, which are reported to occur in 17% of patients[34]. Importantly, in this study, after rechallenge with statins 92% of discontinued patients still used statins one year after the statin-related event[34]. Additionally, multiple meta-analyses demonstrated that in trial settings drug discontinuation due to side effects did not differ among statin- and placebo-treated patients[35,36]. Perhaps negative media attention has played a role in creating a negative perception towards statin treatment, and therefore patients more often prefer not to use them, of more often experience side-effects (nocebo effect)[37,38]. Therefore,

when symptoms suggestive of side-effects are reported, it is important to discontinue and rechallenge treatment to verify whether the reported complaints are indeed side-effects, such as statin-induced myopathy, or might be transient in nature and not attrituble to statins. In patients with LDLc not on target, and in whom statin treatment is not preferable, the relatively new class of PCSK9 inhibitors, which have the potential to reduce LDLc by 32-71%, might be an alternative option[39,40].

Additionally, we found that adherence to the Dutch guidelines for healthy lifestyle[41,42] was very poor, and did not differ among patients on target LDLc or not on target LDLc. Only 6% of patients had a BMI ≤25kg/m$^2$. Adherence to the guideline on physical activity was 59%, although we question the validity of the used subjective questionnaire after we found a big difference between results from subjective and objective assessment of physical activity (see also **Chapter 6**). Shockingly, only 7% of the population are used to ingest a sufficient amount of vegetables (≥200 g/day). Additional guideline adherence rates were: fruit (≥200 g/day, 28%); legumes (once per week, 59%); nuts (≥15 g/day, 14%); dairy products (2-3 portions/day, 19%); fish (once per week, 36%); tea (2-3 cups/day, 8%), red meat (<45 g/day, 12%); processed meats (no consumption, 2%); cooking fats (choose soft margarines, liquid cooking fats, and vegetable oils, 66%); sweetened beverages (no consumption, 34%); and salt (<6 g/day, 12%). It is important to note that there was little difference between dietary intake in our population and that reported in the Dutch general population[43-45], illustrating that non-adherence to dietary guidelines is a population-wide phenomenon.

There is good opportunity to improve LDLc through lifestyle measures, particularly in those with statin intolerance or a personal preference not to use statins. Previously it has been demonstrated that dietary changes can reduce LDLc by 13-30% in non-statin users[46-49]. Unfortunately, little data is available on the effect of dietary changes on LDLc in patients already on statin treatment. The efficacy of statin treatment in LDLc reduction might overshadow the positive effects of lifestyle intervention in treatment of dyslipidaemia. It has been previously suggested by stakeholders of nutritional research in the Netherlands that the high efficacy of pharmacological therapy might be one of the reasons that lifestyle intervention has taken a back seat in clinical care[50]. However, adopting a healthy lifestyle has beneficial effects not only on dyslipidaemia, but also on blood pressure, insulin resistance and direct CVD risk[51-57]. Therefore, although the effect of lifestyle intervention on a single factor, such as in this case LDLc, might be more modest than that of a pharmacological agent, the total CVD risk reduction of effective and lasting lifestyle change is substantial and should not be overlooked.

**Low target achievement and high pharmacological treatment resistance in glycaemic control in a real-life setting of T2DM: Important opportunity for lifestyle intervention.**

Glycaemic control is an important part of risk management in patients with T2DM. Adequate glycaemic control (HbA1c <53 mmol/mol) is mostly associated with a substantial risk reduction of microvascular complications, and to a lesser extent also of CVD[58-61]. However, the worldwide pooled average target achievement for HbA1c in patients with T2DM is only 43%[62]. In **Chapter 4**, we investigated the prevalence of ideal HbA1c target achievement in DIALECT-1, and performed an integrated assessment of both lifestyle and pharmacological treatment, to identify opportunities for improving ideal HbA1c target achievement.

We found that the ideal HbA1c target was reached in only roughly one-third of the patients (36%). Patients who did not reach the target had a higher amount of insulin resistance compared to patients who did reach the target, reflected by a longer duration of diabetes, higher percentage of insulin use and higher dosage of daily used insulin. In parallel, patients who used the highest amount of insulin, also had the highest BMI and waist-to-hip circumference. These findings suggest pharmacological treatment resistance in patients who do not achieve the ideal HbA1c, and that these patients are in a vicious circle of insulin resistance, insulin treatment and weight gain. Therefore, further increasing insulin dosage to improve glycaemic control is not the preferable option, as this aggravates the downward spiral. Instead, the better choice would be increasing insulin sensitivity, which can be achieved through lifestyle intervention (i.e. weight loss, increasing physical activity and adopting a healthy diet), or the use of the relatively new blood glucose lowering drugs SGLT-2 inhibitors and GLP-1 analogues.

Comparable to BP and LDLc management, in almost all patients not on target HbA1c, there is opportunity to improve glycaemic control through lifestyle intervention. However, the urgency for lifestyle intervention in HbA1c treatment is higher than for the other two CVD risk factors, as there is a high degree of pharmacological treatment resistance. In a number of patients, lifestyle intervention might be the only option to improve glycaemic control. Lifestyle opportunities for improvement of HbA1c were weight loss, increasing physical activity, and adopting a healthy diet, as all these interventions can improve insulin sensitivity[63-66].

Regarding pharmacological therapy, special mention should be made of SGLT-2 inhibitors and GLP-1 analogues, as large trials have shown that these agents do not only reduce HbA1c, but also reduce weight, reduce long-term cardiovascular risk, and do not cause hypoglycaemia[67-69]. In contrast, many of blood glucose lowering drugs induce weight

gain (i.e. sulfonylureas and insulin), and therefore, further increase insulin resistance. Pharmacological blood glucose lowering treatment that reduces weight, not only improves blood sugar in the short term, but in the long term may also slow the progression of insulin resistance in the course of T2DM. As, in the Netherlands, reimbursement for these agents when insulin is also used has become available since 2016 (SGLT-2 inhibitors) and 2017 (GLP-1 analogues), their use has become more prominent in the last few years. Especially in patients who do not achieve the ideal HbA1c target despite very high dosages of insulin, use of SGLT-2 inhibitors and GLP-1 analogues should not be overlooked. Additionally, in patients with inadequate glycaemic control treated with non-insulin drugs, use of these agents may be preferable to starting insulin treatment, as they can support lifestyle changes made by the patients.

### Do magnesium intake and magnesium status protect against coronary heart disease in T2DM?

Next to traditional risk factors for CVD, such as BP and LDLc, it is also important to study emergent risk factors. In the general population, low dietary magnesium (Mg) intake, which is associated with poor overall diet quality[5], and low Mg status have been associated with increased risk of coronary heart disease (CHD). However, in T2DM, where Mg handling is deranged, little is known on the association between Mg and CHD. Therefore, in **Chapter 5** we studied the associations between dietary Mg intake, 24h urinary Mg excretion, and plasma Mg concentration and prevalent CHD in DIALECT-1.

We demonstrated that higher dietary Mg intake and Mg status are associated with a lower prevalence of CHD in T2DM. The robustness of our findings was supported by the fact that the association between higher Mg and lower CHD prevalence was consistent for dietary Mg intake, 24h Mg excretion and plasma Mg concentration.

In clinical practice, Mg deficiency is often overlooked as a contributor to disease, as it is not part of routine measurements. Nevertheless, Mg is the second most abundant intracellular cation in the human body, and is essential for more than 300 enzymatic reactions. In the last decade, interest in the negative effects of low magnesium intake and/or status has fuelled multiple investigations on the link between magnesium and adverse health outcomes. In T2DM, adequate Mg intake and status might be even more important that in the general population, as hypomagnesemia is highly prevalent in T2DM, occurring in 14-48% of patients, compared with 3-15% in subjects without T2DM[70,71]. This could be mediated through the bidirectional association between Mg and insulin resistance: low Mg intake/status increases insulin resistance, and insulin resistance increases renal Mg losses[70-74]. In accordance, previous studies demonstrated favourable effects of Mg supplementation on BP, cholesterol profile and fasting blood glucose in hypomagnesemic

patients with T2DM[72,75]. We are the first to illustrate that in T2DM, adequate Mg intake and Mg status might be protective for CHD. Our findings emphasize that Mg deficiency should not be overlooked in clinical practice and support the hypothesis that higher Mg intake is beneficial in T2DM.

In addition, we demonstrated that it is important to consider micronutrient intake, in this case Mg, as a part of a complete dietary pattern. When exploring whether dietary source of Mg intake was also associated with lower CHD risk, we found that Mg derived from vegetables was particularly protective for CHD. However, in this population total vegetable intake was low, and vegetables only contributed for 3% to the total Mg intake. Taken together, increasing Mg intake through increasing intake of Mg rich vegetables (for example 200g spinach, or 100g rucola lettuce and 2 avocados per day) seems promising.

**Substantial difference between results from objective and subjective assessments of physical activity in patients with T2DM - the case for incorporating objective measurements in routine care.**

Cardiovascular risk management and T2DM guidelines across the globe recommend sufficient moderate to vigorous physical activity (MVPA) in all subjects, mostly ≥150 minutes of MVPA per week[76-78]. However, methods to properly monitor physical activity are not incorporated into these guidelines. In clinical practice, measuring MVPA is mostly limited to subjective assessment; through interviewing or questionnaires. It is unclear to which extent subjective assessment of performed MVPA provides adequate information on which treatment can be based. Therefore, in **Chapter 6** we compared subjective with objective assessment of physical activity.

The study was performed in 50 patients included in DIALECT. Subjective assessment of physical activity was done with the Short Questionnaire to ASsess Health enhancing behaviour (SQUASH), and objective assessment was done with a wearable accelerometer (Fitbit Flex). We found that according to the SQUASH, guideline adherence (≥150 minutes of MVPA per week) was 40%. In contrast, according to the Fitbit measurements, guideline adherence was 2%, and 14% when including non-registered activities (i.e. cycling, swimming, fitness). The number of weekly minutes MVPA measures with the SQUASH and Fitbit were strongly correlated, however, the total number of minutes MVPA measured with the SQUASH was substantially higher.

To our knowledge, this is the first study that compared subjective assessment with objective measurements of physical activity guideline adherence in patients with complicated type 2 diabetes mellitus. We show that the subjective measurement of MVPA is inappropriate in the vast majority, as most patients overestimated their physical activity (figure

1). Why are these findings important? Although physical activity recommendations are present in guidelines, currently there is no strong recommendation as to its monitoring[79]. This is somewhat surprising. For comparison: it would be hard to conceive of blood pressure management without blood pressure measurements.

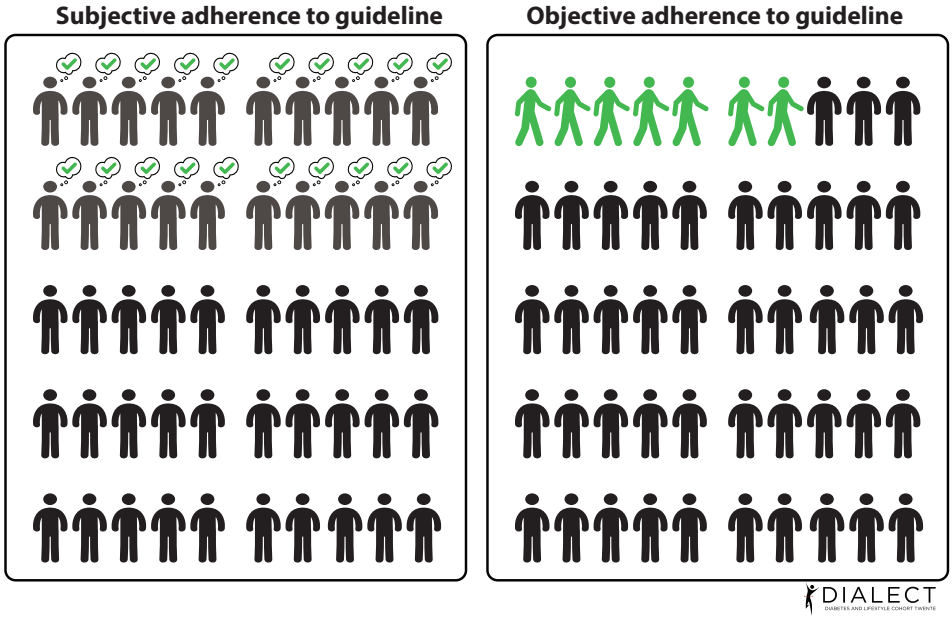


**Figure 1**. Difference in adherence to the guideline on physical activity between self-reported weekly physical activity and objectively measured physical activity.

Since (low) physical activity is considered a "risk factor" or target for treatment, this urges for routine measurement. First, it is important to properly identify the individuals at risk. In current practice, it is unlikely that efforts to increase physical activity are undertaken in patients who seem to be already sufficiently active, so this opportunity will be missed in many cases. Additionally, demonstrating objectively measured results can increase patients' awareness of unperceived physical inactivity. Furthermore, follow-up of measurements is necessary to evaluate the effect of treatment. Objective measurements can help to monitor persistence of increased activity. As our data illustrate that patients overestimate their activity, we propose that objective measurements of activity should be considered as the standard evaluation of physical activity.

## Part 2. Optimizing pharmacological treatment in cardiovascular risk management: neurohumoral activation in T2DM and chronic kidney disease.

### Aldosterone, a new player in cardiovascular disease.

Next to improving quality of health care, it is still important to investigate pathophysiological mechanisms behind increased CVD risk, so that pharmacological treatment targeting these pathways can be optimized. In **Chapter 7** we reviewed the non-classical effects of aldosterone, and mineralocorticoid receptor antagonism. Aldosterone is classically known for inducing sodium and water retention in the distal tubules of the kidney. However, in the last decades it has become known that aldosterone also induces pro-inflammatory and pro-fibrotic effects on end organs, such as the heart, the kidneys and the vasculature[80,81]. Interestingly, the deleterious effects of aldosterone seem most pronounced in the presence of a high sodium diet[82]. In albuminuric kidney disease, inhibition of the renin-angiotensin-aldosterone system (RAASi), of which aldosterone is the end product, is the mainstay of treatment to reduce albuminuria and prevent renal function decline. However, during RAASi, aldosterone levels initially decline, but in 50% of patients return to, or even exceed pre-treatment levels (aldosterone breakthrough). It remains unclear whether aldosterone breakthrough during RAASi is associated with adverse outcomes, especially when sodium intake is high.

Aldosterone can be antagonized by mineralocorticoid receptor antagonists (MRA), such as spironolactone and eplerenone. In heart failure, MRA is an important part of treatment, as it reduces mortality and morbidity[83,84]. In kidney disease, MRA has been demonstrated to be effective in reducing residual albuminuria during RAASi[85]. In addition, in patients with resistant hypertension, MRA is an effective additional treatment option[13]. However, long-term studies on the effect of MRA on CVD risk are still lacking. It would be of great interest to investigate whether MRA in patients with aldosterone breakthrough, with and without a high sodium intake, can reduce cardiovascular events.

### Men have higher aldosterone, extracellular volume and blood pressure then premenopausal women.

Aldosterone is the end product of the RAAS and is a main effector agent in volume homeostasis. Although higher plasma aldosterone levels have been reported in men than in women, no formal investigations were performed on whether gender differences in volume homeostasis also exist. Therefore, in Chapter 8 we investigated plasma aldosterone levels, extracellular volume (ECV) and blood pressure (BP) in healthy men ($n$=18) and premenopausal women ($n$=18), during a regular (200 mmol/24h) and low (50 mmol/24h) dietary sodium intake. In addition, we studied the plasma aldosterone response to exogenous angiotensin II infusion. Study conditions were highly standardized, as aldosterone

levels are dependent on sodium intake and, in women, on phase of the menstrual cycle. We found that plasma aldosterone concentration, ECV, and BP were significantly higher in men than in premenopausal women during a high sodium intake. Higher aldosterone in men was previously reported, however we are the first to describe this under highly standardized conditions[86,87]. Moreover, we found that the adrenal response to exogenous angiotensin II infusion was lower in men than in women in both sodium intakes. This suggests that higher endogenous angiotensin II activity or sensitivity contributes to higher aldosterone levels in men.

To our knowledge, we are the first to report higher ECV and BP in men under these standardized conditions. The combination of higher aldosterone, and higher ECV is suggestive of increased volume retention due to higher aldosterone in men. However, intervention studies with MRA could provide additional evidence to substantiate this mechanism. In addition, as the higher ECV in men was paralleled by a higher BP, this might suggest that aldosterone mediated volume retention is associated with higher blood pressure in men. It should be noted that differences in gonadal hormones and sympathetic regulation might also add to gender differences in blood pressure[88-90]. In hypertensive subjects, blood pressure is commonly higher in men than in premenopausal women[89,91]. We demonstrate that this BP difference holds true in non-hypertensive subjects. Although results from our study in normotensive subjects cannot be directly extrapolated to hypertensive subjects, comparable gender differences in RAAS activity and volume homeostasis might exist in hypertensive subjects. Therefore, our research provides important insight into possible gender differences in the pathophysiology of essential hypertension. Further research on this topic is important, as our findings could have implications for aldosterone-mediated increased CVD risk in men. Additionally, our findings could indicate that pathophysiological mechanisms behind hypertension might differ between men and women, and therefore, men and women might respond differently to different types of antihypertensive agents[92].

### Plasma aldosterone is higher in patients with lower renal function, despite RAASi, and is associated with higher blood pressure.

In chronic kidney disease (CKD), volume derangement and hypertension are common. An inverse association between aldosterone and renal function has been described, possibly adding to hypertension and CVD risk in CKD. However, the effects of RAASi on aldosterone levels in CKD are unknown. Therefore, in **Chapter 9** we investigated the determinants of plasma aldosterone in CKD, during a regular and low sodium intake, both during placebo treatment and during RAASi. In addition, we studied the association between plasma aldosterone levels and blood pressure.

To this end we performed a post-hoc analysis in a previously performed randomized controlled cross-over trial with 6-week study periods of 1) placebo treatment, 2) RAASi (losartan 50mg/day), and 3) RAASi + HCT (hydrochlorothiazide 25mg/day), all during a regular (200 ± 10 mmol $Na^+$/day) and low (89 ± 8 mmol $Na^+$/day) dietary sodium intake.

We found that in CKD, plasma aldosterone is inversely associated with creatinine clearance, similarly during placebo and during RAASi treatment. The correlation was independent of plasma renin activity or plasma potassium. In a comparable crossover trial in CKD patients with single and dual RAASi and a standardized sodium intake, we found a similar association between lower renal function and higher aldosterone, similarly during single and dual RAASi. Furthermore, those with higher baseline aldosterone had a higher blood pressure, which subsided after treatment with RAASi and intensive removal of extracellular volume with diuretics and dietary sodium restriction.

The mechanism behind higher aldosterone in patients with a lower renal function is unknown. We hypothesized that the link between renal function and aldosterone could be PTH mediated due to the bidirectional association between PTH and aldosterone. However, in new evidence, MRA did not decrease PTH in primary hyperparathyroidism[93]. Also, in post-hoc analyses we performed of intervention trials in T2DM and CKD patients with the vitamin D agonist paricalcitol, plasma PTH levels were reduced but aldosterone levels did not change (data not presented in this thesis)[94,95]. Alternatively, increased aldosterone levels might be part of neuro-endocrinological derangement found in CKD. It is known that circulating cortisol levels are higher in CKD[96-98]. Increased ACTH might also increase aldosterone excretion.

Nevertheless, our data suggest that in CKD patients, plasma aldosterone is increased, and is associated with a higher blood pressure. Therefore, MRA could be a good option to treat hypertension in CKD and could possibly reduce long-term CVD complications of high aldosterone. However, further research is needed to elucidate the mechanism behind higher aldosterone in CKD, and the long-term efficacy of MRA.

### Intracellular cortisol production is higher in T2DM and inversely associated with renal function.

Metabolic similarities between patients with T2DM with Cushing's syndrome have fuelled the hypothesis that relative hypercortisolism, increased cortisol exposure while plasma cortisol is in the normal range, might occur in T2DM[99]. Dysfunction of the enzymes 11beta-hydroxysteroid dehydrogenase type 1 (11β-HSD1; regenerates cortisone to active cortisol) and 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2; reduces cortisol to inactive cortisone) in T2DM and CKD have sporadically been reported, with an

increase in intracellular cortisol exposure in both conditions[100-104]. In **Chapter 10** we investigated whether 11β-HSD activities differ between patients with T2DM and healthy controls, and whether there is an association between eGFR and 11β-HSD activities in patients with T2DM.

Total body 11β-HSD activity and 11β-HSD2 activity were assessed in DIALECT-1 and in healthy controls, by the determining the ratios of 24h urinary excretion of (tetrahydrocortisol+allo-tetrahydrocortisol)/tetrahydrocortisone and cortisol/cortisone respectively. We found that total body 11β-HSD and 11β-HSD2 activities were shifted towards higher intracellular cortisol production in patients with T2DM, compared to healthy controls. In addition, lower eGFR was associated with higher intracellular cortisol production by both total body 11β-HSD, and 11β-HSD2. To our knowledge, we are the first to report on 11β-HSD activities in patients with T2DM and renal function impairment.

Our finding of higher 11β-HSD activities in T2DM patients versus healthy controls could imply that derangement of 11β-HSD activities may play a role in the development of T2DM. In mice, increased activity of 11β-HSD1 in the liver has been associated with reduced insulin sensitivity[105]. Additionally, also in humans higher 11β-HSD1 activity has been reported in patients with T2DM versus obese subjects without T2DM[100,101]. It has been well established that obesity-related low-grade inflammation, characterized by an increase in macrophages and cytokines, plays an important role in the development of T2DM[106]. Findings on increased 11β-HSD1 in T2DM suggest that derangement of intracellular cortisol production might be an alternative pathway through which inflammatory pathways might be associated with the development of T2DM. As answering this question is outside of the scope of our data, future studies should be performed to investigate the role of 11β-HSD activities in the development of T2DM, and whether inhibition of intracellular cortisol generation might halt increasing insulin resistance in at-risk subjects.

Additionally, the association between higher intracellular cortisol production in patients with lower renal function raises some important questions. First, in the last decade, pharmacological compounds which inhibit 11β-HSD1 activity have been under development. Preclinical trial demonstrated that these agents had beneficial effects of all parameters of the metabolic syndrome (i.e. glycaemic regulation, blood pressure, dyslipidaemia), and even demonstrated modest weight loss[107]. However, effects on these components were relatively small. Our data suggest that 11β-HSD1 inhibitors might have increased efficacy in patients with T2DM and renal function impairment, and therefore, further research on this topic is warranted. Second, 11β-HSD2 activity was lower in patients with reduced renal function. Dysfunction of 11β-HSD2 is associated with increased MR activation

by cortisol, the syndrome of apparent mineralocorticoid receptor excess. Therefore, in addition to the higher serum aldosterone concentration in patients with renal function impairment (**Chapter 9**), dysfunction of 11β-HSD2 might also lead to increased MR activation in CKD. As MR activation is associated with hypertension, pro-inflammatory and pro-fibrotic effects on the kidneys (**Chapter 7**), dysfunction of 11β-HSD2 may play a role in the development or progression of diabetic nephropathy. This is further supported by the efficacy of MR antagonism of reducing residual albuminuria in CKD (**Chapter 7**). Future studies are necessary to further characterize the role of 11β-HSD2 dysfunction in T2DM, and diabetic nephropathy.

# General discussion

### Integrated assessment of pharmacological and lifestyle management: a new method of evaluating treatment and basis for shared decision making in CVRM.

In **Part 1** of this thesis, systematic integrated assessment of both pharmacological and lifestyle intervention provided unique insight into incorporation of cardiovascular risk management in a real-life setting of secondary care T2DM. This new method of assessing CVRM demonstrated for the first time, that for each individual treatment target (BP, LDLc and HbA1c), opportunities for increasing target achievement are different with respect to the potential of lifestyle and pharmacology intervention (figure 2). Thus, for dyslipidaemia, increasing pharmacological treatment with pharmacological agents provides the strongest potential for improving target achievement, as treatment resistance to statins rarely occurs. In BP treatment on the other hand, there is less opportunity to achieve the target by pharmacological treatment alone, as resistance to pharmacological intervention is a problem in approximately one-third of this population of patients with long-standing T2DM. Finally, for glycaemic control, insulin resistance is high in those who do not achieve the target, and thus, it does not seem logical or fruitful to aim for improved target achievement by increasing insulin use. Instead, there is an urgent need for improvement of lifestyle. Also, there is a need for the development of additional pharmacological agents such as SGLT-2 inhibitors and GLP1-analogues, that reduce insulin resistance, do not cause weight gain, and reduce long term CVD risk. Importantly, lifestyle intervention has the potential to improve achievement of all treatment targets simultaneously, in part due to the fact that lifestyle guideline adherence was very low in the great majority of patients, and in part due to the fact that lifestyle improvement will likely exert pleiotropic beneficial effects. These pleiotropic effects are not only supposed to include increasing chances of target achievement for BP, LDLc and HbA1c, but also a direct reduction of the risk of CVD and multiple types of cancer through other – still miscellaneous, but firmly acknowledged – mechanisms[14-18,108]. Importantly, intensive lifestyle intervention has also been shown to significantly improve outcomes related to depression and quality of life in obese subjects and patients with T2DM[109,110]. Moreover, considering growing treatment resistance over time, in particular for hyperglycaemia and BP, addition of lifestyle intervention might be the only option to reach target values in some patients. It should be noted that in overall CVRM in these high-risk patients, lifestyle intervention should go hand in hand with pharmacological treatment, in an integrated fashion.

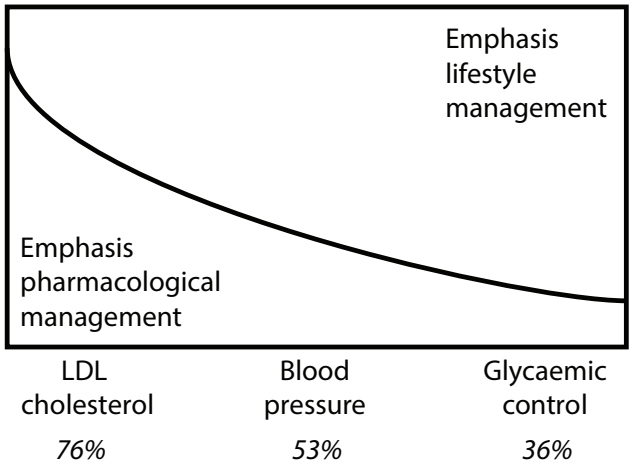


**Figure 2.** Opportunities for improving target achievement are different for each treatment target. For LDL cholesterol, the opportunity for improvement is emphasis on increasing pharmacological management, for blood pressure, the emphasis should be placed on both lifestyle and pharmacological management, and for glycaemic control, the emphasis should be placed on predominantly lifestyle management. The percentage of patients on target was lower for targets with a higher necessity for lifestyle management.

Integrated assessment of both pharmacology and lifestyle provides a complete picture of the current treatment situation, clearly highlighting where intervention might be warranted. The advantages may become clearer in an example. Without integrated assessment, classical medicine would assess a patient as follows: male, 53 years, not on target BP of 150/80 mmHg, currently treated with three different antihypertensive agents on the maximum dosage. In this assessment, the lifestyle situation of the patient is missing, and it is unclear where the opportunities for improvement lie. An integrated assessment would, however, include such information and the patient would be presented as follows: male, 53 years, not on target BP of 150/80 mmHg, currently treated with three different antihypertensive agents on the maximum dosage, salt intake of 15g per day, vegetable intake of 50g per day, BMI of 35 kg/m$^2$, and weekly moderate to vigorous physical activity of 10 min. Now it is immediately clear which lifestyle habits may maintain the high BP despite considerable use of antihypertensives, and the opportunities for lifestyle intervention are clear (reduce salt intake, weight loss, increase physical activity). In addition to providing a complete picture for the caregiver, such an overview can also be the basis for shared decision making in CVRM, where pros and cons and expected treatment effects of each management option, be it pharmacological or lifestyle, can be estimated and discussed (figure 3).

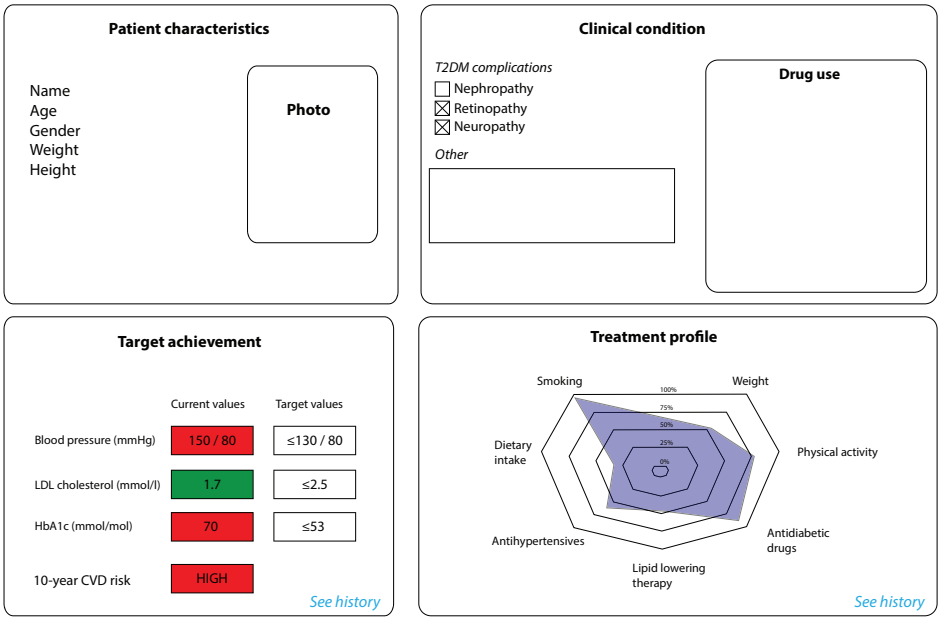


Figure 3. Example of a patient vignette for cardiovascular risk management, incorporating target achievement and current lifestyle and pharmacological management. Treatment modules support shared decision making for the next step in treatment, by illustrating expected treatment effects on blood pressure, LDL cholesterol, HbA1c and 10-year cardiovascular disease risk of the intervention. Treatment modules can be active or passive, and illustrate which targets the patient and physician are currently working on. Numbers of treatment effects shown in the figure are fictional. BP blood pressure; HbA1c glycated haemoglobin; CVD cardiovascular disease; RAASi renin-angiotensin-aldosterone-system inhibition.

**Current CVRM in routine care: high degree of pharmacological treatment, and low adherence to lifestyle guidelines.**

In DIALECT, we found that pharmacological treatment was implemented reasonably well, however lifestyle guideline adherence was very low, suggesting that pharmacological treatment has become main focus in treatment. Our studies highlight that increasing lifestyle care is a missed opportunity in CVRM. With respect to resistant hypertension, addition of an additional pharmacological agent can reduce BP by 4-9 mmHg[13]. Alternatively, lifestyle interventions can reduce systolic BP by varying amounts: -1 mmHg per 1kg weight reduction; -11 mmHg for a diet rich in fruits, vegetables, whole grains and low-fat dairy products; -5/6 mmHg for a low dietary salt intake <6 g/day; -5/8 mmHg for high dietary potassium intake of 3.5-5 g/day; -5/8 mmHg for increasing physical activity; -4 mmHg for moderation of alcohol intake[111].

The question rises why lifestyle management has taken a back seat in secondary health care, despite the fact that it is beneficial. Although we did not investigate the causes of low achievement of lifestyle guidelines in this population, we speculate that several patient/interpersonal factors and healthcare factors might contribute towards low lifestyle adherence (Table 1). Patient/interpersonal factors could be the following: low health literacy due to cognitive factors; inability to remember the details of recommendations correctly; suboptimal physician-patient relationship; low patient involvement in decision making; negative patient attitude towards the recommendation; depression or other psychological disorders[112]. Important to note here, is the attitude towards lifestyle habits. Possibly, lifestyle is considered a private matter, and adopting a healthy lifestyle may be considered the patients' own responsibility. In line, non-achievement of lifestyle guidelines could be expressed as non-compliance, implicating patient unwillingness to improve their lifestyle, rather than lack of patient ability due to the factors described above. Our research clearly shows that currently, the large majority of patients does not adhere to the lifestyle guidelines. We believe that a supportive attitude towards lifestyle, where professional healthcare supports and empowers patients to adopt a healthy lifestyle, might benefit lifestyle guideline adherence more than considering lifestyle purely a private matter.

Apart from patient/interpersonal factors, different healthcare factors associated with low lifestyle adherence have been previously suggested[50]. The first of which, is the current organization of health care, including the current financing structure. There is a lack of incentive for financing of lifestyle intervention in routine care. While pharmacological therapy is invariable reimbursed by health insurances, in basic coverage the only reimbursed lifestyle treatment is three hours of dietician counselling per year, which is too little for proper lifestyle change. In addition, research into the efficacy of pharmacological treatment and the development of new agents can be funded by pharmaceutical

companies, while research into lifestyle lacks structural funding. Other healthcare factors associated with low lifestyle adherence are: lack of evidence from randomized controlled trials in patient populations; difficulty to achieve long-lasting behavioural changes in combination with lack of embedded motivational behavioural intervention in routine care; lack of cooperation between different health care disciplines; low quality and contradictory reports in the media; and insufficient communication between the media and health professionals, and between health professionals themselves; highly variable degrees of dietary knowledge among caregivers. We propose that lack of monitoring of lifestyle habits in routine care also plays an important role, because it is difficult to treat something that is not measured.

**Table 1**. Patient and healthcare barriers that reduce adherence to lifestyle recommendations in routine care

| ***Patient/interpersonal factors*** |
|---|
| Low health literacy |
| Difficulty to remember recommendations |
| Poor physician-patient relationship |
| Low patient involvement in decision making |
| Patient has negative attitude towards recommendation |
| Depression or other psychological disorders |
| ***Healthcare factors*** |
| Current organization of health care, especially the current financing structure |
| Lack of evidence from randomized controlled trials in patient populations |
| Difficulty to achieve long-lasting behavioural changes and no motivational behavioural intervention in routine care |
| Lack of cooperation between different health care disciplines; |
| Low quality and contradictory reports in the media |
| Insufficient communication between the media and health professionals, and between health professionals themselves |
| Highly variable degrees of lifestyle knowledge of nurses, nurse specialists and physicians |
| No routine monitoring of lifestyle in routine care |

### Lifestyle intervention is performed in temporary programs, outside of routine care.

When lifestyle intervention is applied, it is often in the form of temporary programs, outside of routine care, with a set duration of often up to 24 months. Such programs have shown beneficial effects on weight, blood pressure, HbA1c and lipids in the short term[19,113-115], however, the majority of patients have difficulty to maintain the health lifestyle after finalization of the intervention program, limiting the efficacy of the intervention[116-118]. A meta-analysis of 10 studies with lifestyle-based weight interventions

in patients with T2DM demonstrated a pooled weight loss of -5.33 kg (95% confidence interval -7.33, -3.34 kg). Studies ranged from 16 weeks to 9 years[119]. Important to note was that only one of these studies also assessed whether weight loss was maintained after the end of the intervention, and in this study body weight increased after the intervention stopped[120]. Another meta-analysis on the sustainability of interventions for diabetes prevention in adults, demonstrated an initial significant risk reduction of 39% (relative risk 0.61; 95% confidence interval, 0.54-0.68) for diabetes incidence with lifestyle intervention[121]. After the washout period, ranging from 2-52 weeks after the intervention, risk reduction decreased to 29% (relative risk 0.71; 95% confidence interval, 0.55-0.92), demonstrating the importance of continued lifestyle monitoring and intervention.

Another setback of interventional programs is that patient selection occurs often, reducing external validity. In a real-life setting, patients are highly heterogenous, differing in amount of co-morbidity and motivational stage. A long term multifaceted approach towards behavioural change might be able to reach more patients than a one-size fits all interventional approach[122].

### Look towards the future of CVRM in routine care - incorporation of individualized lifestyle and pharmacological treatment.

So, what are the implications of our research for future CVRM care? Increasing adherence of lifestyle recommendations in routine care was the greatest opportunity we identified after integrated assessment of pharmacological and lifestyle intervention. We propose that to improve lifestyle guideline adherence, all aspects of lifestyle intervention should become part of routine clinical secondary CVD prevention. In routine clinical care, long-term lifestyle monitoring and intervention can be applied, and the intervention can be tailored to the specific preferences and needs of the individual patients, instead of a one-size-fits-all approach found in interventional programs. In secondary prevention, the individual patient is already known and face-to-face contact is regular, which provides a strategic advantage over primary prevention. We propose the following changes in health care organization as the first steps to facilitate increasing lifestyle intervention into routine care.

First, low time-consuming, convenient and affordable methods to objectively measure lifestyle habits should become available in clinical practice. E-Health options can provide opportunities to measure lifestyle habits, providing direct and objective feedback for the patient and the professional[123,124]. We demonstrated the importance of such objective measurements in **Chapter 5**. Regarding physical activity, wearable activity trackers can monitor steps and heart-rate 24 hours per day and estimate both physical inactivity and moderate-to-vigorous physical activity. After integration of data derived from such

activity trackers into the ICT structure of the health care organization, the health care provider can quickly review the results, comparable to reviewing results from routine laboratory measurements. Unfortunately, diet is more difficult to objectively assess. In the field of nephrology, 24h urinary excretion of various substances, such as urea, sodium and potassium, is traditionally used to objectively estimate dietary intake. Patients have indicated that these measurements help them achieve targets of e.g. sodium intake[122]. But not all relevant information can be derived from such objective measurements; to address total dietary intake and dietary patterns, other methods are necessary. The tools we currently have available are 24h dietary recall, dietary record and food frequency questionnaires[125]. However, these methods are time-consuming for the patients, suffer from recall bias, and require expert dietary knowledge to analyse the data. Attempts to standardize and simplify registration of food intake by e-health solutions, such as mobile applications to register diet, have not yet overcome these setbacks of traditional methods[126]. Therefore, it is of paramount importance that an easy tool will be developed to objectively monitor dietary intake. It should be noted, that when using E-Health options, measures should be taken to assure that privacy of personal data is guaranteed. Objective assessment of lifestyle habits has multiple benefits: increase patients and doctors' awareness of unhealthy habits; facilitate self-monitoring; provide motivational feedback and ongoing follow-up for continued progress and maintenance of behaviour change; and timely identification of relapses to previous unhealthy behaviour, allowing for just-in-time interventions.

Second, caregiver knowledge on lifestyle and psychological concepts of behavioural change should be increased, and multidisciplinary communication should be improved. Patients who are under treatment in secondary care, have contact with different caregivers, including nurses, nurse practitioners, physicians and dieticians. In general, lifestyle and psychology of behavioural change are not part of caregiver education, with the result of highly variable levels of knowledge on these subjects per caregiver[50]. When knowledge on what a healthy lifestyle encompasses, and how lifestyle affects disease is different per caregiver, patients might receive conflicting advices. Next to that, in routine care, lifestyle counselling is rarely done using motivational interview techniques[127]. However, in the field of psychology there is abundant research and knowledge regarding behavioural change[128-130]. Caregivers in routine care should be educated on psychological concepts of behavioural change, so that these can be used to support patients. Lastly, communication between dieticians and other caregivers is often infrequent, leading to the situation that the medical treatment team is unaware of diet interventions performed by the dietician, reducing efficacy. When lifestyle intervention would become part of routine care, the whole treatment team would become aware of the current lifestyle situation and current ongoing interventions. Followingly, the team can give uniform advices and can

provide motivational support according to psychological concepts of behavioural change. In such a situation, it is also important to establish the roles of different caregivers. As in the current care structure, physicians only have 10 minutes per patient visit, perhaps it might be more feasible if dieticians or nurse practitioners should take the lead in lifestyle interventions. During regular care visits, physicians can follow the progress of behavioural change, and provide encouragement for patients.

Lastly, methods to evaluate the quality of lifestyle management in routine care should be developed. The goal here is not to gather new knowledge on the effects of lifestyle on CVD, but rather to investigate how we can improve current lifestyle management. The use of quality improvement projects, instead of randomized controlled trials (RCT), is much more practical to use for this end[131]. In the last 50 years randomized controlled trials (RCT) have been pivotal to scientific progress and substantiation of concepts and treatment. However, in health care improvement, the role of RCTs is less pronounced[132]. The real-life relevance of results derived from RCTs is limited, due to low external consistence of results after extensive patient selection. In addition, RCTs are costly, and funding is difficult to find for lifestyle intervention RCTs. Finally, e-health technologies are constantly under development, and therefore to study their application, a flexible iterative study design is necessary, instead of the rigid RCT design, so that the used technology can be upgraded during the course of the study. Quality improvement projects are often based on cyclically implemented changes, in which self-evaluation is integrated[126]. After each cycle, helpful interventions can be selected, while unsuccessful efforts can be discarded. Therefore, implementation of a quality control project is more flexible and cheaper than the traditional RCT. To introduce such a project, monitoring and measuring of the target outcome, in this case lifestyle habits, is pivotal, underlining the importance of adding lifestyle measurements to routine care. However, quality improvement projects also have some setbacks, such as low quality of data analyses and reporting, often only single-centre implementation and lack of understanding the mechanics of change[133]. For pharmacological treatment, diverse quality control systems already exist, and it has been illustrated that these improve the quality of care and patients' outcomes[134,135]. As in this moment no such systems exist for lifestyle treatment, we propose that the introduction of quality control cycles in lifestyle management will improve the implementation and execution of lifestyle management in routine care.

### Optimization of pharmacological therapy: the role of aldosterone and neurohumoral activation in CVD prevention.

Next to improving lifestyle intervention, optimization of pharmacological treatment can be an important strategy to improve CVD risk reduction. The urgency of lifestyle intervention is greatly determined by the efficacy of pharmacological treatment. For

example, for HbA1c treatment, pharmacological treatment resistance is frequent, and therefore there is great urgency for lifestyle intervention, while for LDLc treatment this is not the case. In the last decade, it has become increasingly known that efficacy of pharmacological agents can greatly vary between individuals, a phenomenon to which pharmacogenomics and inflammatory state have been mentioned to contribute[136,137]. Therefore, it is important to identify on an individual level which drugs might be most effective to treat the patient (i.e. personalized medicine). In **part 2** of this thesis we investigated neurohumoral activation in healthy adults, patients with CKD and patients with T2DM, and focused in particular on regulation of aldosterone and 11β-HSD activities. These data may be used to pinpoint patient that might benefit from treatment efficacy of agents that interfere with aldosterone and 11β-HSD activities (i.e. mineralocorticoid receptor antagonists and 11β-HSD1 inhibitors).

Aldosterone is a promising target in CVD prevention strategies (**Chapter 7**). Patients with primary hyperaldosteronism, in which aldosterone production in the adrenal glands is increased, have a higher risk of CVD than patients with essential hypertension, also after treatment[138,139]. However, research into regulation of aldosterone is complicated due to the fact that aldosterone should always be viewed in the light of current volume status and sodium intake, where plasma aldosterone is higher when volume status or sodium intake is low. Abnormal high aldosterone for a given sodium intake, or inappropriately high aldosterone, might be more detrimental than physiologically increased high aldosterone due to volume depletion.

Our research has provided insight into regulation of aldosterone in highly standardized conditions. In **Chapter 8** we found that plasma aldosterone was higher in men then in women, especially during a high sodium intake. In addition, in **Chapter 9** and **Chapter 10**, we found that patients with renal function impairment have higher plasma aldosterone (independent of RAASi, and reduced 11β-HSD2 activity, which are both highly suggestive of increased MR activation in CKD. Next to that, in **Chapter 8** and **Chapter 9**, there were signs that higher aldosterone for a given sodium intake was paralleled by higher blood pressure. These findings are relevant, because often in routine clinical practice targets on blood pressure are not reached, especially in those with albuminuric kidney disease (**Chapter 2**). While our data suggest that MRA might be particularly effective to reduce blood pressure in patients with CKD, further intervention studies are necessary to investigate this hypothesis. In addition, future studies should be performed to elucidate whether inappropriately high aldosterone in men, and high aldosterone in combination with reduced 11β-HSD2 activity in CKD patients, are also associated with a greater risk of developing CVD, and whether MRA can reduce this risk.

Lastly, in **Chapter 10**, we also found that 11β-HSD activities are shifted towards excess higher intracellular cortisol production in T2DM and in diabetic nephropathy, compared to healthy controls. Future studies should investigate whether treatment effect of 11β-HSD1 inhibitors in patients with T2DM is higher in those who also have renal function impairment.

Taken together, our findings illustrated that for a given sodium intake, aldosterone is higher in men than premenopausal women, and in patients with renal function impairment. Additionally, in T2DM, lower renal function is associated with higher intracellular cortisol production, which might be associated with increased activation of the MR. Therefore, neurohumoral activation in kidney disease and T2DM might contribute to hypertension and increased long-term cardiovascular risk. However, currently there is no evidence to support standard MRA use in CVD prevention in these populations. Nevertheless, we suggest addition of MRA should be considered in CKD patients with high blood pressure or residual albuminuria, and patients with T2DM with high blood pressure or albuminuria.

# Conclusion

In this thesis we studied opportunities for improving CVD prevention in routine clinical T2DM care, by integrated assessment of both lifestyle and pharmacological factors. We found that in routine secondary T2DM care, targets of blood pressure, LDL cholesterol and HbA1c are reached by half, three-quarters and one-third of the population, respectively. Furthermore, in T2DM, adequate dietary magnesium intake should also be addressed, as higher intake is associated with a lower prevalence of coronary heart disease. Finally, when assessing lifestyle habits, it is important use objective measures, as people have the tendency to overestimate health-enhancing behaviour. Overall, pharmacological treatment is implemented reasonably well in clinical practice, while lifestyle guideline adherence is very low in almost all patients. Therefore, there is great opportunity to improve outcomes through lifestyle intervention. We propose that incorporation of lifestyle monitoring and treatment in routine clinical care can improve lifestyle guideline adherence. To this end, objective measurements of lifestyle habits should become readily available, multidisciplinary communication between caregivers should be improved, caregivers should be educated on lifestyle and on psychological concepts of behavioural change, and quality control cycles for lifestyle management should be developed.

In addition, we studied the potential role of neurohumoral activation in CVD prevention, in order to optimize pharmacological treatment. We found that for a given sodium intake, plasma aldosterone was higher in men than in premenopausal women, and that this was paralleled by a higher extracellular volume and higher blood pressure in men. Further research is needed to study gender difference in therapeutic efficacy of different classes of antihypertensives. Additionally, in patients with renal function impairment, aldosterone was higher, and 11β-HSD2 was lower, both suggestive of increased mineralocorticoid receptor activation in kidney disease. As mineralocorticoid receptor activation increases blood pressure and exerts pro-inflammatory and pro-fibrotic effects on different end-organs, future studies should be performed to investigate efficacy of mineralocorticoid receptor antagonism on reducing blood pressure and reducing long-term CVD risk patients with (diabetic) kidney disease.

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# APPENDICES

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# APPENDIX

## Dutch Summary
Nederlandse Samenvatting

# Nederlandse samenvatting

Bijna de helft van de Nederlandse volwassenen heeft overgewicht, of zelfs obesitas, zo luidden de laatste cijfers van het Rijksinstituut voor Volksgezondheid en Milieu. Ter vergelijking, in 1990 was dit nog een-derde van de bevolking. Deze enorme stijging van overgewicht is in de gezondheidszorg terug te zien in een toename van het aantal leefstijl-gerelateerde aandoeningen zoals suikerziekte (type 2 diabetes mellitus) en chronische nierziekte. Patiënten met deze aandoeningen lopen een hoog risico op het ontwikkelen van hart- en vaatziekten, die hun kwaliteit van leven ernstig kunnen beperken of zelfs kunnen lijden tot vroegtijdig overlijden. Het voorkómen van hart- en vaatziekten is dus een van de belangrijkste kerntaken in de moderne geneeskunde, en met name in de begeleiding van hoog-risico-groepen zoals diabetespatiënten. Leefstijl-management en medicamenteuze behandeling zijn daarvoor de twee pijlers.

Wij onderzochten een tweetal strategieën om de bestaande behandeling van hoog-risico patiënten te verbeteren. Ten eerste hebben we systematisch de bijdrage geïnventariseerd van medicamenteuze en niet-medicamenteuze behandelingen bij het halen van streefwaarden voor bloeddruk, cholesterol en glucoseregulatie in patiënten met type 2 diabetes mellitus. We vonden dat streefwaarden worden bereikt bij respectievelijk slechts 53%, 76% en 36% van de patiënten, terwijl de meeste patiënten dagelijks al 5 of meer pillen gebruiken, en daarnaast insuline gebruiken. Vervolgens keken we, aan de hand van de behandelrichtlijnen, waar ruimte voor verbetering is. Voor wat betreft medicamenteuze behandeling van bloeddruk is er in twee-derde van de patiënten die de streefwaarde niet bereikten mogelijkheid om het aantal bloeddrukverlagende middelen te verhogen, waarbij er in de overige patiënten sprake is van therapie-resistentie. Bij cholesterolbehandeling is er weinig resistentie voor therapie, bij 92% van de patiënten niet op streefwaarde kan de medicatie worden geïntensiveerd. Bij glucoseregulatie bleek therapieresistentie een belangrijk probleem, maar slechts in 43% van de patiënt niet op streefwaarde is er nog ruimte voor intensivering van de medicatie. Voor wat betreft beter leefstijl management is er over het gehele spectrum volop mogelijkheid om de behandeling te verbeteren. Slechts 5% van de patiënten heeft een gezond gewicht, slechts 3% eet dagelijks voldoende groente en fruit, en slechts 14% van de patiënten voldoet aan de richtlijn gezond bewegen. Dit illustreert dat beter leefstijl-management een harde noodzaak is voor een betere behandeling van de hoog-risico-patiënt, en meer geprioriteerd moet worden in de behandeling van leefstijl-gerelateerde aandoeningen. Het is echter gebleken dat het lastig is om leefstijl goed te integreren in de spreekkamer van de arts, mede omdat tot nu toe leefstijlgewoonten moeilijk objectief te meten zijn, en dit een tijdrovende kwestie is; en hoe kan iets worden behandeld wat niet meetbaar is? Deels is leefstijlinformatie af te leiden uit routinegegevens uit de zorg: in onze studies gebruikten

we daarvoor het lichaamsgewicht, en de zoutuitscheiding in de 24-uurs urine, maar dit biedt helaas geen volledig beeld. Nieuwe eHealth technologieën, zoals stappentellers kunnen de oplossing bieden om leefstijl snel en objectief meetbaar te maken. Wij hebben reeds laten zien dat zodanige objectieve metingen onmisbaar zijn in het inschatten van daadwerkelijke gewoonten van de patiënt; met een vragenlijst naar beweging schatte 40% van de patiënten in dat zij voldoende bewegen, maar objectief gemeten met een stappenteller was dit maar 14%.

Hiernaast hebben wij onderzoek gedaan naar strategieën om bestaande medicamenteuze therapieën effectiever te kunnen toepassen. Zodoende hebben we onderzocht of verschillen tussen patiënten aanknopingspunten kunnen bieden voor therapiekeuzes meer passend bij het individu, oftewel personalized medicine. Hierbij hebben we ons gericht op de negatieve effecten van aldosteron en overstimulatie van de aldosteronreceptor. Van oudsher staat aldosteron bekend als een hormoon dat leidt tot het vasthouden van water en vocht op het moment dat het lichaam een vochttekort heeft. Recente onderzoeken laten echter zien dat verhoogd aldosteron kan leiden tot ontsteking en verbindweefseling in de bloedvaten, het hart en de nieren, en zo dus het risico op hart- en vaatziekten en nierziekten kan vergroten. In onze studies vonden we dat mannen in vergelijking tot vrouwen een hoger aldosteron hebben, en dat patiënten met een slechte nierfunctie ook een verhoogd aldosteron hebben. Daarnaast vonden we dat patiënten met diabetes, en patiënten met een verminderde nierfunctie, een verhoogde activatie van de aldosteronreceptor door cortisol hebben door een verminderde afbraak van cortisol in eindorgaancellen. Dit kan er op wijzen dat mannen, en nierpatiënten mogelijk extra baat hebben bij medicamenteuze aldosteronremming ter bescherming tegen hart- en vaatziekten en bescherming tegen achteruitgang van de nierfunctie.

Concluderend is de boodschap van dit proefschrift dat het voorkómen van hart- en vaatziekten in hoog-risico-patiënten verder geoptimaliseerd kan worden door enerzijds het medicatieregime verder te intensiveren, en aan te passen aan het pathofysiologische profiel van de patiënt, en dat er anderzijds volop ruimte is voor verbetering van leefstijlgewoonten. Het routinematig toepassen van objectieve leefstijlmetingen kan hierbij een zinvolle stap zijn om leefstijlmanagement een meer prominente rol te geven in de zorg.

# APPENDIX

## Acknowlegdements
Dankwoord

# Dankwoord

Het einde van een promotietraject is een belangrijk moment om stil te staan bij alle gebeurtenissen van de afgelopen jaren. Op dit moment aangekomen realiseer ik mij maar al te goed hoeveel mensen hebben bijgedragen aan mijn promotie en het schrijven van dit proefschrift. Daarom wil iedereen bedanken die mij in enige vorm dan ook heeft bijgestaan met het uitvoeren van het onderzoek. Bepaalde personen wil ik hier in het bijzonder nog even noemen.

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# APPENDIX

About the author

# About the author

Christina Maria Gant was born in Amersfoort the Netherlands, on the 6th of July 1989 to a Dutch mother and Spanish father. She grew up in Terschuur and attended grammar school, the Johan van Oldenbarnevelt Stedelijk Gymnasium, in Amersfoort.

In 2007 she moved to Groningen to study Medicine at the University of Groningen. She completed her bachelor with an honours degree after participation in the Junior Scientific Masterclass program. During this time, Christina investigated antimicrobial resistance in *Streptococcus pneumoniae* in the La Fé Hospital in Valencia, Spain. She performed her scientific internship on the molecular mechanisms behind the before mentioned antimicrobial resistance. This work was published, and she presented it on an international congress. During her studies Christina was an active member of the International Federation for Medical Students' Association (IFMSA); she organised student exchanges and represented the division of Groningen in several international meetings.

In 2011 Christina started her medical internships in Ziekenhuisgroep Twente (ZGT) Almelo/Hengelo. Here, she was the treasurer of the organizing committee of the ZGT Benefietgala; an event which raised €28,000 for child abuse. During her Masters' studies Christina became involved in the DIAbetes and LifEstyle Cohort Twente (DIALECT) at the Department of Nephrology of the ZGT, in collaboration with the University Medical Centre Groningen. To further her ambition herein she was accepted to the MD/PhD program of the Junior Scientific Masterclass Groningen, and she performed her PhD research in ZGT between 2015 and 2018. During this time, she was project manager of DIALECT, coordinated the PRIORITY intervention study, and was co-initiator of the Diabetes and Lifestyle Coaching Project Twente (DELICATE) which was awarded the Pioneers of Healthcare Innovation Fund and the prize for best diabetes idea of 2017 by the Diabetesfonds.

Christina recently returned to her roots and is currently working as a resident in internal medicine at the Meander Medisch Centrum in Amersfoort. Her ambition is to become an internist, with a special focus for the use of eHealth in treatment of lifestyle related diseases. Her hobbies include catching up with friends around the country, drumming in a band, visiting Spain and reading.

# APPENDIX

List of publications

# List of publications

**Gant CM**, Mensink I, Binnenmars SH, van der Palen JAM, Bakker SJL, Navis GJ, Laverman GD. Weight course in the DIAbetes and LifEstyle Cohort Twente - an 18-year observational study. Manuscript in progress.

Oosterwijk MM, Soedamah-Muthu SS, Geleijnse JM, Bakker SJL, Navis GJ, Binnenmars SH, **Gant CM**, Laverman GD. High Dietary Intake of Vegetable Protein is Associated with Lower Prevalence of Renal Function Impairment: Results of the Dutch DIALECT-1 Cohort. Under review at Kidney International Reports.

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